Title: Destruction of Spores on Building Decontamination Residue in a Commercial Autoclave

Running Title: Destruction of Spores on BDR in a Commercial Autoclave

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ABSTRACT

The U.S. Environmental Protection Agency (EPA) conducted an experiment to evaluate the effectiveness of a commercial autoclave at treating simulated building decontamination residue (BDR). The BDR was intended to simulate porous materials removed from a building deliberately contaminated with biological agents such as *Bacillus Anthracis* (anthrax) in a terrorist attack. The purpose of these tests was to assess whether the standard operating procedure in a commercial autoclave provides sufficiently robust conditions to adequately destroy bacteria spores bound on the BDR. This study investigated the effect of several variables related to autoclaving BDR, including time, temperature, pressure, item type, moisture content, packing density, packing orientation, autoclave bag integrity, and autoclave process sequence. The test team created simulated BDR from wallboard, ceiling tiles, carpet, and upholstered furniture, embedded with 10^6 population *Geobacillus stearothermophilus* biological indicator (BI) strips and thermocouples to obtain time/temperature profile data associated with each BI strip.

Results indicate that a single standard autoclave cycle did not effectively decontaminate the BDR. Autoclave cycles of 120 minutes at 31.5 psig/275 °F and 75 minutes at 45 psig/292 °F effectively decontaminate the BDR material. Two standard autoclave cycles of 40 minutes and 31.5 psig/275 °F run in sequence proved to be particularly effective, probably because the second cycle's evacuation step pulled the condensed water out of the pores of the materials, allowing better steam penetration. Results also indicate that packing density and material type of the BDR in the autoclave can significantly impact on the effectiveness of the decontamination process.

INTRODUCTION

In the event of a terrorist attack on a building where biological weapons (BW) such as *Bacillus Anthracis* (anthrax) might be used, much of the porous material in the building may be shipped for disposal after decontamination activities. These materials are collectively termed “building decontamination residue” (BDR). Although the BDR may be disinfected or decontaminated prior to shipment, it may need additional decontamination to ensure the contaminating agent has been destroyed, or because of heightened political sensitivities (e.g., a stigma attached to the waste), may need to be handled as if it were still contaminated. There are no mandated action levels for residual spores in such materials, and the emergency response personnel or on-scene coordinators typically work with relevant state regulators to make determinations of what constitutes proper BDR disposal. Much of this BDR might be tightly packed and possibly wet. EPA has initiated a research program to investigate issues related to the proper disposal of BDR (Lemieux, 2005).

Autoclaves are commonly used to effectively treat regulated medical waste by exposing the waste to steam at elevated pressures and temperatures for extended periods of time (e.g., 31.5 psig, 275 °F, and 40 minutes) (Fleming et al., 1995). However, it is unknown whether the standard operating procedure in a commercial autoclave will provide sufficient time/temperature/pressure to adequately destroy residual bacteria spores bound on BDR.

The primary objective of this study (Sieber and Osborne, 2005) was to establish whether standard operating conditions at a commercial medical waste autoclave are sufficient to destroy bacteria spores potentially found on BDR, and if not, what modifications to the standard operating procedure could be recommended to assure complete spore destruction. The secondary objective of this study was to investigate the time/temperature dependence of *Geobacillus stearothermophilus* spore destruction as a function of autoclave operating conditions and BDR composition. *Geobacillus stearothermophilus* was chosen because it is widely available and commonly recommended (Technical Assistance Manual, 1998) for moist heat sterilization validation.

MATERIALS AND METHODS

Autoclave Description

EPA conducted these tests on March 4-6, 2005 at the Healthcare Environmental, Inc. facility located in Oneonta, New York, approximately 90 miles from Albany. The facility can treat up to 84 tons of medical waste per day using two identical autoclaves 8 feet in diameter and 32 feet long, which accept large metal bins (80 inches by 54 inches by 69 inches) on rollers. Each autoclave (Bontech Model 886) can process six bins, with a total mass of approximately 3,000-4,000 pounds per cycle.

The State and Territorial Association on Alternate Treatment Technologies developed a document (Technical Assistance Manual, 1998) to establish a framework or guideline that defines medical waste treatment technology efficacy criteria and delineates the components required to establish an effective state medical waste treatment technology approval process. The report recommends that all medical waste treatment technologies achieve six logs or greater microbial inactivation of mycobacteria and four logs or greater reduction of spores. Approximately 32 states incorporate the STAATT criteria for the treatment of regulated medical waste or biohazardous waste. Effective December 2005, the STAATT criteria now also apply to autoclave technologies. While BDR may not be classified at regulated medical waste, a commercial autoclave rather than a bench-scale autoclave, was investigated because of the quantities of BDR that may be generated in the event of a biological contamination.

The nominal autoclave operating cycle time is 40 minutes plus cool-down time to prepare for subsequent loads. At the start of each cycle, the autoclave is sealed and air is evacuated for 3 minutes using a vacuum pump to approximately -10 psig. Steam is then injected to reach and maintain the desired operating pressure and temperature, typically within approximately 5 minutes. The nominal operating conditions during the cycles are 31.5 psig and 275 °F. Steam is injected through three ports at the top of the autoclave, located at the front, center, and rear. The steam is injected over distributor plates to cause turbulent, disbursed steam flow throughout the autoclave. At the end of each cycle, the steam is evacuated by again pulling vacuum.

Testing Approach

Autoclave performance was judged based on two parameters: real-time measurements from thermocouples and viability of BI test strips containing 10^6 spores of *Geobacillus stearothermophilus* embedded within each load of simulated BDR material tested. The testing comprised a series of test runs at different conditions in one of the facility autoclaves (Unit A1).

For each test run, 24 thermocouples were embedded in the BDR material to record the time/temperature profile at different locations within the load. Additional control thermocouples not embedded in BDR recorded the temperature inside and outside the autoclave (for data completeness and as an additional diagnostic for temperature measurement instrumentation operation). The thermocouple wires passed into the autoclave through a custom flange plate with a Swagelok bulkhead fitting packed with high temperature RTV-silicone sealant (see Figure 1). Real-time temperature measurements were monitored and recorded at each sampling point using a GEC Instruments Model S27TC temperature measurement system and Type "T" thermocouples. Temperatures were recorded to the hard disk at approximately 10-second intervals.

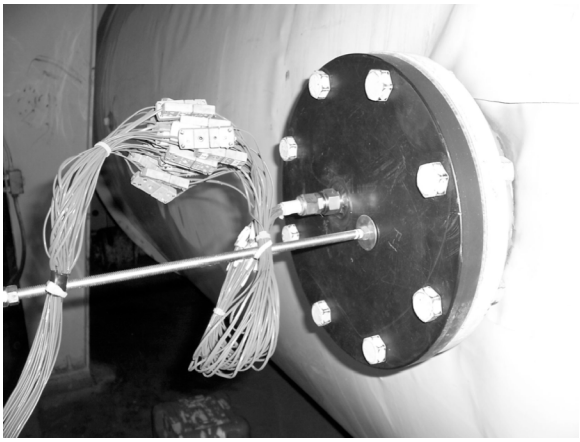

Figure 1. Bulkhead Flange for Temperature Measurements

A BI pouch was paired with a thermocouple at each test location (see Figure 2). Each BI pouch contained two *Geobacillus stearothermophilus* (American Type Culture Collection #7953, Lot #3167091, expiration January 2007, D_{121} Value of 1.5 minutes, $D_{132.2}$ Value of 0.14 minutes) indicator strips, labeled 'A' and 'B', encased in a GS Medical Packaging self-seal pouch (#222100). Each BI strip contained a 10^6 population of spores on Schleicher & Schuell filter paper (#470) encased in a glassine peel-open envelope. Raven Biological Laboratories, Inc. (Raven) manufactured the BI strips and assembled the BI pouches. After the test, the A strips were analyzed for a growth/no-growth indication using the United States Pharmacopeia (USP) Viable Spore Count procedure (United States Pharmacopeia, XXV). The strips were removed from their respective pouches, transferred into Tryptic Soy Broth with bromocresol purple indicator, and incubated at 55-60ºC for 7 days. If the A strips showed viable spores, a population assay was performed on the corresponding B strips using the USP Biological Indicator (spore strip) Population Determination (United States Pharmacopeia, XXV). The population was determined after 24-hour incubation at 55-60ºC in Tryptic Soy Agar. Three types of control BI test pouches were also used in the test: BI test pouches fully exposed to the autoclave conditions (but not embedded within BDR), BI test pouches packaged and handled similarly to other BDR but not autoclaved, and duplicate BI test pouches (i.e., two pouches placed next to each other within the BDR). Figure 3 illustrates placement of the fully exposed controls.

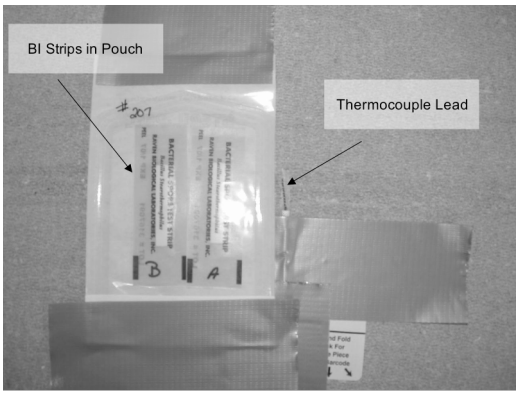

Figure 2. BI Pouch

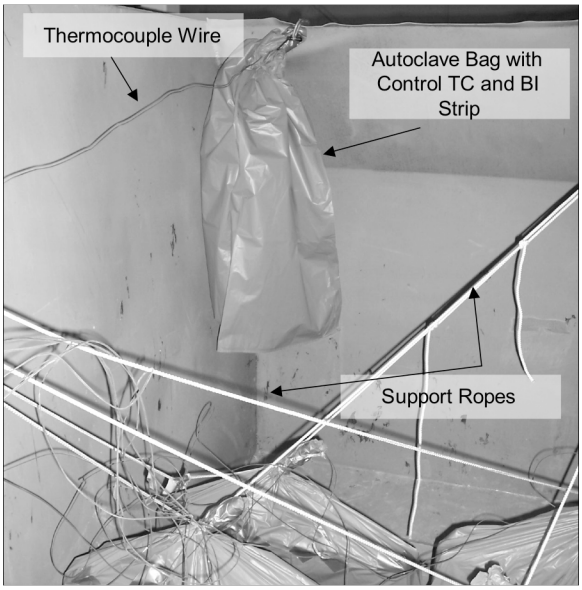

Figure 3. Control Placement (carpet and ceiling tile shown)

The following variables were identified as having a potential impact on penetration of hot steam into the BDR and therefore impact spore destruction capability:

- Item type (wallboard, ceiling tile, carpeting, upholstered furniture);
- Moisture content of autoclaved materials (wet, dry);
- Autoclave packing density (loose, dense);
- Packing orientation (horizontal, vertical);
- Opening autoclave bags prior to cycle;
- Autoclave temperature/pressure (31.5 psig/275 °F, 45 psig/292 °F);
- Time in autoclave (up to 2 hours); and
- Impact of multiple sequential autoclave cycles.

The test matrix presented in Table 1 was designed to investigate the effects of each of these variables.

Item Types

Unpainted wallboard (LaFarge regular grade ½" thick drywall) was cut into approximately 2-feet-by-2-feet sections. Sample BDR bags were formed by placing five 2-feet-by-2-feet sections face to face in autoclave bags. Wallboard was

Table 1. Test Matrix

Run	Item Type	Packing Arrangement	Pressure/ Temperature	Time (min)
1	Mixed	Loose, Horizontal	31.5 psig/275 °F	120
2	Wallboard	Dense, Horizontal	45 psig/292 °F	120
3	Carpet	Loose, Dense, Large Roll	31.5 psig/275 °F	120
4	Mixed (incl. sofa)	Loose, Horizontal	45 psig/292 °F	75
5	Mixed	Loose, Vertical	31.5 psig/275 °F	40 (x 2 sequential runs)
6	Mixed	Loose, Vertical, Open Bags	31.5 psig/275 °F	40 (x 2 sequential runs)

Mixed: wallboard, ceiling tile, and carpet

tested both wet and dry. In this context, dry refers to as-is condition at ambient humidity with no additional moisture added. Wetted samples were submerged in a tank of water for 30 seconds and placed on a drain rack for 5 minutes prior to being placed in the bag. Dry test bags weighed approximately 34 pounds, and wet bags weighed approximately 37 pounds. Samples were double bagged in 1.8 mil polypropylene autoclave bags (to represent likely practices that would be found at an emergency response), and the bags were individually goose-necked and taped shut using duct tape. A section of nylon rope was attached to the gooseneck to allow test personnel to easily and safely load and unload the bags from the autoclave bins. Three types of wallboard bags were created. Some test bags (called "1-sample" bags) were assembled with one thermocouple and one test strip pouch placed together, between the second and third wallboard section. Other test bags (called "3-sample" bags) were assembled with three thermocouples paired with three test strip pouches placed between the first and second, second and third, and fourth and fifth wallboard sections. Additional bags were made without thermocouples and BIs, to be used as fillers.

Ceiling tiles (Armstrong Contractor Series 5/8" thick Ceiling Panels Model #942) were cut into approximately 2-feet-by-2-feet sections. Samples were prepared similarly to wallboard; however, bags contained nine 2-feet-by-2-feet sections placed face to face. Dry test bags weighed approximately 23 pounds, and wet bags weighed approximately 31 pounds. "1-sample" bags contained one thermocouple and one test strip pouch placed together, between the fourth and fifth ceiling tile section. "3-sample" bags contained three thermocouples paired with three test strip pouches placed between the second and third, fourth and fifth, and seventh and eighth ceiling tile sections. Additional bags were made without thermocouples and BI test pouches, to be used as fillers.

Carpet (Mannington Nepenthe II Blue commercial grade carpeting with Nylon 6,6 fibers) was tested in two configurations, small and large rolls. For small rolls, the carpet was cut into strips 26 inches wide by 20 feet long, representing how carpet would most likely be removed from a building. Some samples were soaked with a hose-end sprayer. After soaking, samples were rolled and placed on end to allow free-flowing water to drain. Small rolls were bagged in a similar manner to wallboard and ceiling tiles. Dry test bags weighed approximately 26 pounds, and wet bags weighed approximately 40 pounds. As a worst case, larger sections of carpet 6 feet wide and 24 feet long were also tested. Large rolls were only prepared wet, and weighed approximately 200 pounds, the maximum size that could be reasonably handled by two workers. Large rolls were wrapped in polypropylene and all seams sealed with duct tape. For the small carpet rolls, 1- and 3-sample bags were prepared. "1-sample" bags contained one thermocouple and one test strip pouch placed together, at the approximate mid-point of the radius of the

carpet roll. "3-sample" bags contained three thermocouples paired with three test strip pouches placed two laps in from the top, at the mid-point of the radius, and two laps from the center of the carpet roll. Additional bags were made without thermocouples and BI test pouches, to be used as fillers. The autoclave bins at either end of the group of bins placed in for each run were filled with uninstrumented BDR materials to provide thermal mass and minimize the impact of any cold spots within the autoclave.

To represent upholstered furniture, a dry, used queen-sized sleeper sofa was autoclaved in Run 4. Four thermocouple and test strip pouches were paired and embedded at the following locations in the sofa: one sample each was inserted in holes cut approximately 6 inches deep in a back cushion and a seat cushion (the holes were then covered with duct tape); one sample was placed within the folded sleeper mattress; and one was placed between the seat cushions. Although surface contamination of upholstered furniture is the most likely scenario, the BI strips were embedded in the upholstered furniture to simulate a worst-case scenario. The sofa was wrapped in polypropylene with all seams sealed with duct tape, and then placed in the autoclave on a sheet of plywood.

Packing Density

Wallboard was tested at two different packing densities. In low-density packing, six bags were placed in a bin, forming a single layer at the base of the bin; some surfaces of all bags were readily exposed to autoclave temperatures. In high-density packing, 23 bags were placed in each bin, forming layers approximately three to four levels deep. In this arrangement, some bags were exposed directly to autoclave conditions while others were buried within the load in the bin. Ceiling tiles were tested only in a low-density arrangement, as described above for wallboard. Runs of densely packed ceiling tiles were deleted from the test matrix because densely packed BDR material could not be brought up to autoclave temperatures within the 120-minute duration specified in the test plan. Carpet was tested in three configurations. Small rolls, approximately 1 foot in diameter and 26 inches long, were placed in bags. Six bags were placed in a bin for low-density packing, and 25 bags were placed in a bin for high-density packing. In addition, a large, intact roll of carpet 6 feet long and approximately 1.5 feet in diameter was tested in one run. Figure 4 illustrates the dense and loose packing arrangements.

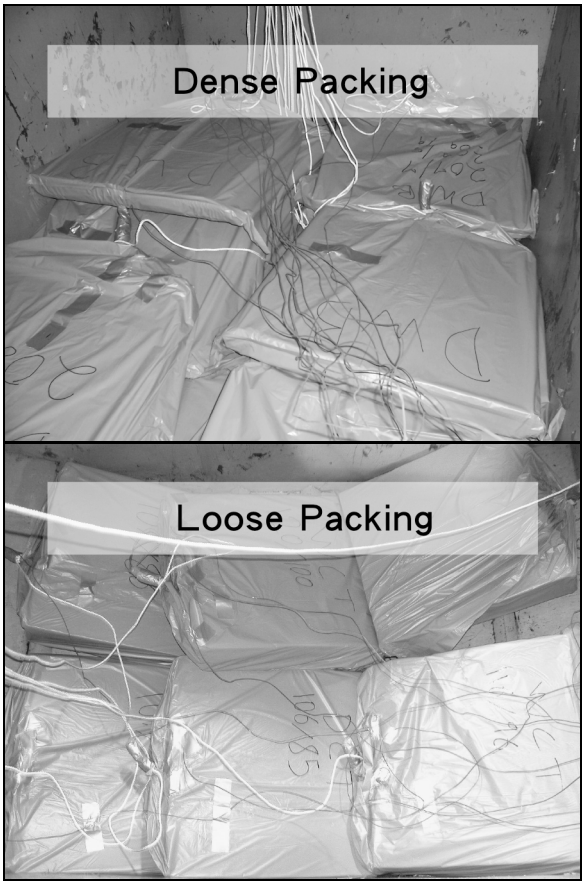

Figure 4. Dense and Loose Packing Arrangements (wallboard shown)

Packing Orientation

Material was tested both lying horizontally (see Figure 4) in the autoclave bins and positioned vertically, with all sides exposed. Material was positioned vertically by tying two ropes to the top of the bag and attaching the ends of the ropes to opposite sides of the autoclave bin wall, as shown in Figure 5. The bags were hung vertically to simulate a rack system to position bags so that all sides were exposed to steam. The test team theorized that hanging the BDR upright would keep it from compressing from its

own weight, and allow steam condensate to drain more easily as it formed. If these hypotheses were correct, they would facilitate steam penetration and more effective heating of the material in the autoclave.

Figure 5. Photograph of Vertical BDR Positioning (wallboard, ceiling tile shown)

Open Bags

All BDR was double-bagged in 1.8 mil polypropylene autoclave bags. The bags were individually goose-necked and sealed with duct tape. This procedure was adopted based on packaging information from the State Department Sterling, Virginia mail facility anthrax cleanup (Army Corps of Engineers, 2002). After autoclaving, some of the bags had clearly ruptured due to temperature and pressure changes. However, in many cases, bag surfaces bubbled and became deformed in the autoclave, but it was not clear if they had fully opened. To test if the bags opening had an effect on decontamination, two bags in Run 5 and all of the bags in Run 6 were opened prior to autoclaving, by slicing open two sides of each bag with a utility knife.

Autoclave Conditions

The test plan initially established a minimum run time of 40 minutes at elevated temperature (275 °F), which is the standard operation of the Healthcare Environmental autoclave. Literature data indicate that holding the material for 15 minutes at 250 °F is required to ensure moist heat sterilization (Boca et al., 2002; Barkley and Richardson, 1994; Gardner and Peel, 1986; Block, 2000). Therefore, the test plan called for extending the run time beyond 40 minutes to achieve a 250 °F temperature target at all, or at least most, embedded thermocouples. Even if the 250 °F target had not been achieved, the test plan established a maximum run time of 120 minutes to enable the autoclave to process multiple test runs each day. Runs 1, 2, and 3 were terminated at 120 minutes, before all thermocouples reached the target temperature. Run 4 was stopped at 75 minutes because the sofa temperature was rising above the temperature of the autoclave, indicating a potential exothermic reaction in the sofa. Because such a reaction and possible associated hazards were not well understood, the run was terminated. The BI strips in the sofa all showed no growth, indicating that the Run 4 conditions were sufficient to decontaminate upholstered furniture. In Runs 5 and 6, two 40-minute runs were conducted in sequence.

Multiple Short Cycles

As steam in the autoclave was evacuated at the end of Runs 2, 3, and 4, the test team observed that, as the vacuum was drawn, most thermocouple readings converged toward a single temperature. It was not known if this resulted from increased turbulence during the post-vacuum cycle, condensed water being drawn out of BDR under vacuum, or some combination of these and other factors. To further investigate this phenomenon, in Runs 5 and 6, two complete normal autoclave-operating cycles were run in succession. Each cycle consisted of a pre-vacuum, steam pressurization, and post-vacuum phase. The cycles were conducted in immediate succession and the autoclave remained sealed throughout both cycles.

RESULTS

Figures 6 through 12 present plots of the time/temperature data recorded during each of the six runs. Some of the figures (Figures 6 and 10) also include readings from the control thermocouple inside the autoclave, the reference thermocouple outside the autoclave, and the autoclave set point pressure/temperature.

Figure 6 shows the time/temperature data from Run 1. Note that in Run 1, there was a significant amount of noise on several of the thermocouple channels, believed to result from condensation accumulating in the thermocouple connection fittings (see Figure 1). After Run 1, the bundle of thermocouple wire outside the flange was positioned so that gravity would prevent condensate from collecting – subsequent runs only showed a minimal amount of noise. It should be noted that although the control thermocouple rapidly approached the autoclave operating temperature, many of the thermocouples never reached the targeted 250 °F temperature. The BI viability measurements on Run 1 were consistent with the temperature measurements (i.e., the BI strips that were at locations where 250 °F was maintained for 15 minutes showed no growth).

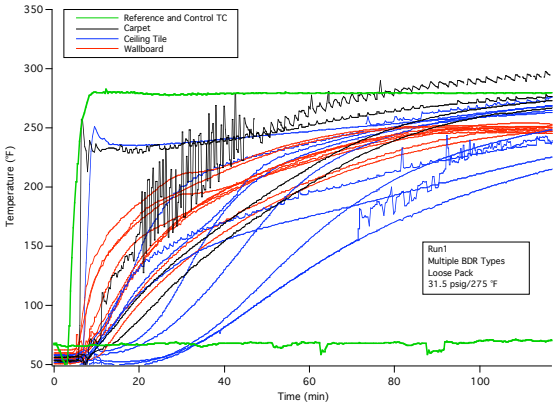

Figure 6. Time/Temperature Data (Loose Packing)

Run 2 (see Figure 7) consisted of subjecting only densely packed wallboard to the highest autoclave pressure/temperature conditions. Again, even at this higher temperature, many of the thermocouples never reached 250 °F. Wallboard is mostly composed of $CaSO_4 \bullet 2(H_2O)$, and loses moisture between 212 and 302 °F (Budavari, 1996). This dehydration step could possibly contribute to the slow heatup times for wallboard, although the bulk density or packing density of the wallboard could also be a factor. The control temperature dropped in Run 2, which was explained later by the fact that the bag containing the control BI strip and thermocouple came loose and fell into the bin, reducing its exposure to the steam. This run was not repeated due to time constraints, but the autoclave facility process monitors exhibited no change at that point in time, which convinced the investigators that the problem was with that one thermocouple. In addition, the temperature signals converged at the end of the run. This observation led to the hypothesis that a second autoclave cycle in sequence might be effective.

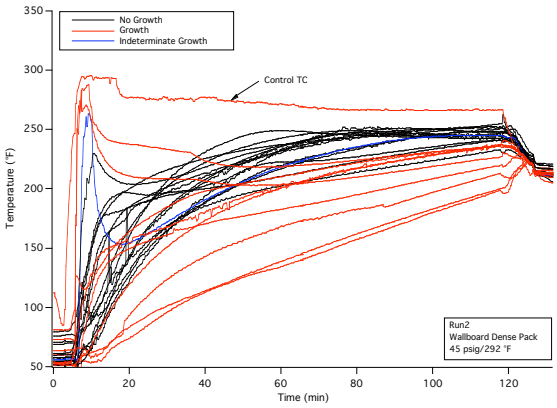

Figure 7. Temperature and Wallboard Spore Viability for Run 2

The data sets depicted in Figure 7 are color coded to indicate if the BIs associated with each thermocouple were viable at the conclusion of Run 2. A viable spore designation was used if growth was found in both the growth/no-growth test and the assay analysis. Decontamination or a no viable spore designation was used if no growth was found in the test with an initial 10^6 spore population. In a limited number of cases, the growth/no-growth test indicated a positive result; however, the subsequent assay analysis measured no quantifiable population (reported result of <100 CFU). These data series are labeled as indeterminate. Note that the sample locations that were maintained at 250 °F for 15 minutes consistently showed no growth on their corresponding BI strips, while most of the sample locations that did not meet that time/temperature still showed growth.

Figure 8 shows the effect of the second autoclave cycle (Run 5), using average temperature data for each individual bag. During this run, bags of various materials were placed upright to maximize exposure during the autoclave cycle, and then a second cycle was run, complete with evacuation and repressurization. At the beginning of the second

autoclave cycle, almost all of the temperatures converged to the operating temperature of the autoclave. It is believed that when the cold, porous BDR material is exposed to the steam during the first cycle, condensate forms in the pores, limiting steam penetration and subsequent heat transfer. With the pores of the material full of water, heat can transfer to the interior of the material mostly through conduction, which is slow, and the steam cannot penetrate very well into the material. At the initiation of the second autoclave cycle, the evacuation step pulls the condensate out of the pores, so that when steam is reapplied, it effectively penetrates the preheated material and reaches the operating temperature of the autoclave. The only thermocouples that did not achieve the necessary temperatures were in the wet carpeting. It was unclear whether the bag with the wet carpet burst open during Run 5. This led to Run 6 being performed with all bags cut open prior to loading. Figure 9 shows the spore viability for Run 5. As before, the samples that did not achieve the necessary time/temperature exhibited residual spore viability.

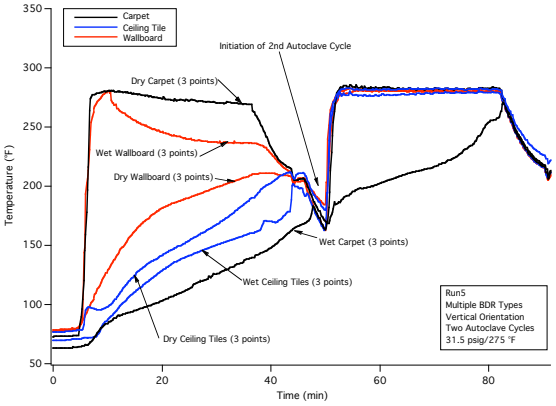

Figure 8. Effect of Second Autoclave Cycle: Impact on Achieving Target Temperature (Averaged Measurements)

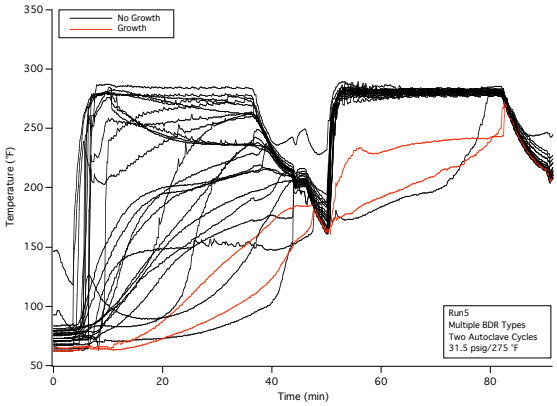

Figure 9. Effect of Second Autoclave Cycle: Impact on Spore Survivability

Figure 10 shows the time/temperature data for Run 6, where the bags were cut open prior to autoclave loading, and two sequential autoclave cycles were performed. In this case, all thermocouples reached the necessary time/temperature to achieve spore destruction, supported by the fact that none of the Run 6 BI strips showed any growth. It was not always obvious whether any given bag ruptured during the cycle, so no definitive conclusions can be made about the effect of changing bag materials as a means to promote bag burst during the autoclave cycle. However, these observations do suggest that packing BDR using bags made from a material that will melt or open during autoclaving might ensure good steam penetration.

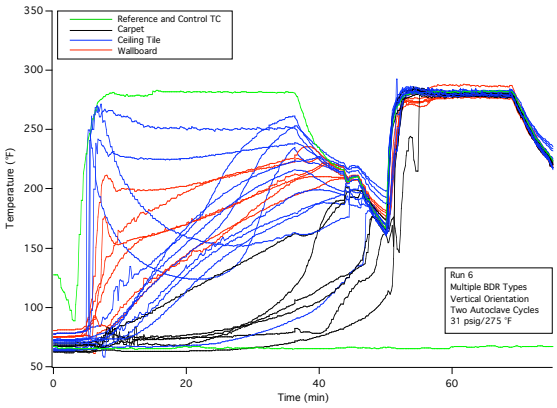

Figure 10. Effect of Second Autoclave Cycle: Impact of Cut Bags

Figure 11 shows the effect of packing density when processing wallboard. Clearly, high-density packing reduces the effectiveness of the

autoclaving. It appears that an autoclave facility processing BDR should minimize packing density so that steam can readily penetrate to each bag in the load.

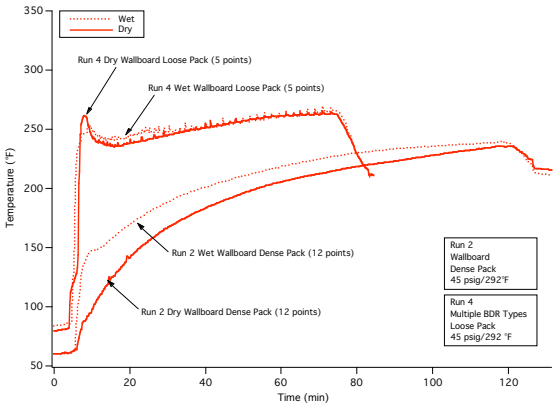

Figure 11. Effect of Packing Density: Wallboard

Figure 12 shows the effect of initial moisture on heating BDR (except for wet carpet, which was not present in Run 1). The wet ceiling tile heated significantly slower than the other BDR item types probably because the micropore structure of the ceiling tiles completely filled with water. The other item types showed similar heating profiles. This supports the hypothesis that initial condensation of steam in the pores of the ambient-temperature BDR limits heat transfer.

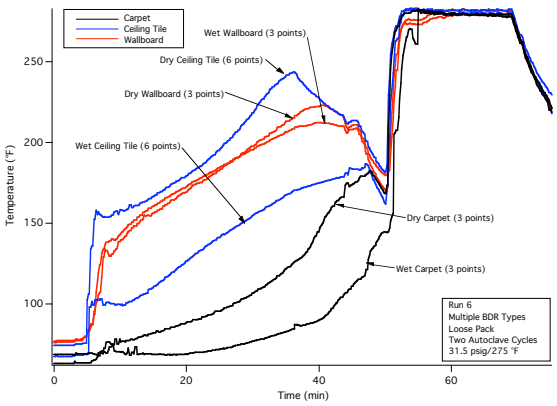

Figure 12. Effect of Moisture Content

DISCUSSION

This paper presents an empirical study to evaluate whether or not moist heat/steam can successfully access all surfaces of porous building materials and furnishings with sufficient potency such that deeply absorbed bacterial spores may be inactivated, and to determine the operational parameters needed to achieve this. While spore strips can present "easily-handled" challenges to this process, it's important to acknowledge that weaponized spores, or even live non-weaponized spores, will likely behave differently. Given the dangerous nature of biological weapon agents, and the severely restricted access and stringent safety protocols necessary to handle live agents, these tests had to be performed on a simulant such as *Geobacillus stearothermophilus*. However, *Geobacillus stearothermophilus* is commonly used as a simulant for agents such as *Bacillus Anthracis*, particularly for studies on technologies utilizing thermal treatment methods to kill the spores (Lemieux et al., 2005). It must be remembered that, in all likelihood, any BDR brought to a disposal facility would have been previously decontaminated and would probably contain very small numbers of viable spores, so testing with BIs that have $1x10^6$ spores represents a worst-case scenario.

Based on the results of these tests, heating the BDR to 250 °F for 15 minutes at the sampling locations resulted in no viable spores. The most effective spore destruction was obtained from:

- Loose packing arrangement;
- Dry BDR material;
- Higher autoclave operating pressure/temperature;
- Multiple autoclave cycles in sequence;
- Bags cut open prior to loading.

The optimal practices for processing BDR in a commercial autoclave are:

- Place BDR so all surfaces are exposed to autoclave conditions;
- Maintain a loose packing arrangement for the materials; and
- Use plastic film bags that allow steam penetration.

The material that was successfully decontaminated included:

- Wet wallboard;

- Dry wallboard;
- Wet ceiling tiles;
- Dry ceiling tiles;
- Dry carpet; and
- Dry upholstered furniture.

Wet carpeting was successfully decontaminated only using cut bags and two sequential autoclave cycles.

Below are conclusions regarding autoclave-operating conditions:

- 120 min @ 31.5 psig/275 °F decontaminated wallboard, ceiling tiles, and dry carpet when loaded as recommended;
- 75 min @ 45 psig/292 °F was sufficient to decontaminate dry upholstered furniture, although there were not sufficient runs with upholstered furniture to determine whether less rigorous conditions would also achieve spore destruction;
- 75 min @ 45 psig/292 °F decontaminated wallboard and ceiling tiles when loaded as recommended; and
- Two standard autoclave cycles of 40 min @ 31.5 psig/275 °F in sequence decontaminated wallboard, ceiling tiles, and dry carpet when loaded as recommended.

The second cycle time may be able to be shortened and still enable destruction of the spores in the BDR. A third cycle may be necessary for wet carpet.

The most important recommendation based on these tests is to run at least two sequential autoclave cycles. The second cycle exhibited a profound effect on the time/temperature profile of the BDR materials being processed. The steam evacuation step between cycles appears to be the critical step for assuring effective decontamination of porous materials in an autoclave.

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