Destruction of Spores on Building Decontamination Residue in a Commercial Autoclave[▽]

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The U.S. Environmental Protection Agency conducted an experiment to evaluate the effectiveness of a commercial autoclave for 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 the tests was to assess whether the standard operating procedure for a commercial autoclave provided sufficiently robust conditions to adequately destroy bacterial spores bound to the BDR. In this study we investigated the effects 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, and embedded in the BDR were *Geobacillus stearothermophilus* biological indicator (BI) strips containing 10⁶ spores and thermocouples to obtain time and temperature profile data associated with each BI strip. The results indicated that a single standard autoclave cycle did not effectively decontaminate the BDR. Autoclave cycles consisting of 120 min at 31.5 lb/in² and 275°F and 75 min at 45 lb/in² and 292°F effectively decontaminated the BDR material. Two sequential standard autoclave cycles consisting of 40 min at 31.5 lb/in² and 275°F 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. The results also indicated that the packing density and material type of the BDR in the autoclave could have a significant impact on the effectiveness of the decontamination process.

In the event of a terrorist attack on a building in which biological weapons, such as *Bacillus anthracis* (anthrax), might be used, much of the porous material in the building may be shipped somewhere for disposal after decontamination. Such material is collectively termed “building decontamination residue” (BDR). Although the BDR may be disinfected or decontaminated prior to shipment, it may need additional decontamination to ensure that the contaminating agent has been destroyed, or because of heightened political sensitivities (e.g., a stigma attached to the waste) the BDR may need to be handled as if it were still contaminated. There are no mandated action levels for residual spores in such material, and the emergency response personnel or on-scene coordinators typically work with relevant state regulators to determine what constitutes proper BDR disposal. Much of the BDR might be tightly packed and possibly wet. The U.S. Environmental Protection Agency has initiated a research program to investigate issues related to the proper disposal of BDR (8).

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 lb/in² and 275°F for 40 min) (6). However, it is not known whether the standard operating procedure for a commercial

autoclave provides sufficient time, temperature, and pressure to adequately destroy residual bacterial spores bound to BDR.

The primary objective of this study (10) was to establish whether the standard operating conditions for a commercial medical waste autoclave are sufficient to destroy bacterial spores potentially found on BDR, and if not, what modifications to the standard operating procedure could be recommended to ensure complete spore destruction. The secondary objective of this study was to investigate the time and temperature dependence of destruction of *Geobacillus stearothermophilus* spores as a function of autoclave operating conditions and BDR composition. *G. stearothermophilus* was chosen because it is widely available and commonly recommended (11) for validation of moist heat sterilization.

MATERIALS AND METHODS

Autoclave description. The Environmental Protection Agency conducted the tests on 4 to 6 March 2005 at the Healthcare Environmental, Inc., facility located in Oneonta, NY, approximately 90 miles from Albany. This facility can treat up to 84 tons of medical waste per day using two identical autoclaves that are 8 ft in diameter and 32 ft long, which accept large metal bins (80 in. by 54 in. by 69 in.) on rollers. Each autoclave (Bontech model 886) can process six bins with a total mass of approximately 3,000 to 4,000 lb per cycle.

The State and Territorial Association on Alternate Treatment Technologies (STAATT) produced a document (11) that established a framework or guidelines that defined efficacy criteria for medical waste treatment technology and delineated the components required to establish an effective state medical waste treatment technology approval process. This document recommended that all medical waste treatment technologies achieve 6 logs or greater microbial inactivation of mycobacteria and 4 logs or greater reduction of spores. Approximately 32 states use the STAATT criteria for the treatment of regulated medical waste

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FIG. 1. Bulkhead flange for temperature measurement.

FIG. 2. BI pouch.

or biohazardous waste. Effective in December 2005, the STAATT criteria are also used for autoclave technologies. While BDR may not be classified as 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 biological contamination.

The nominal autoclave operating cycle time is 40 min 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 min using a vacuum pump to obtain a pressure of approximately -10 lb/in². Steam is then injected until the desired operating pressure and temperature are reached, typically within approximately 5 min, and this operating pressure and temperature are maintained. The nominal operating conditions during the cycles are 31.5 lb/in² 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 that cause turbulent, disbursed steam flow throughout the autoclave. At the end of each cycle, the steam is evacuated again by pulling vacuum on the autoclave.

Testing approach. Autoclave performance was judged based on two parameters: real-time measurements obtained with thermocouples and viability determined with biological indicator (BI) test strips containing 10^6 spores of *G. stearotheophilus* embedded within each load of simulated BDR material tested. The testing comprised a series of test runs with different conditions in one of the facility's 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 the sake of data completeness and as an additional diagnostic for operation of the temperature measurement instrument). The thermocouple wires passed into the autoclave through a custom flange plate with a Swagelok bulkhead fitting packed with high-temperature RTV-silicone sealant (Fig. 1). The real-time temperature was monitored and recorded at each sampling point using a GEC Instruments model S27TC temperature measurement system and type "T" thermocouples. Temperatures were recorded to a hard disk at approximately 10-s intervals.

A BI pouch was paired with a thermocouple at each test location (Fig. 2). Each BI pouch contained two *G. stearotheophilus* (ATCC 7953; lot 3167091; expiration date, January 2007; D_{121} value [i.e., the time it takes for a 1-log kill at 121°C], 1.5 min; $D_{132.2}$ value, 0.14 min) indicator strips, labeled A and B, encased in a GS Medical Packaging self-seal pouch (catalog no. 222100). Each BI strip contained 10^6 spores on Schleicher & Schuell filter paper (catalog no. 470) and was encased in a glassine peel-open envelope. Raven Biological Laboratories, Inc. manufactured the BI strips and assembled the BI pouches. After the test, the A strips were analyzed to determine growth using the United States Pharmacopeia viable spore count procedure (12). The strips were removed from their pouches, transferred into tryptic soy broth with bromocresol purple indicator, and incubated at 55 to 60°C for 7 days. If examination of the A strips showed that there were viable spores, a population assay was performed with the corresponding B strips using United States Pharmacopeia biological indicator (spore strip) population determination (12). The population was determined after 24 h of incubation at 55 to 60°C 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 like other BDR test pouches but not autoclaved, and duplicate BI test pouches (i.e., two pouches placed next to each other in the BDR). Figure 3 shows the position of the fully exposed controls.

The following variables were identified as variables that had a potential impact on penetration of hot steam into the BDR and therefore had an impact on the ability to destroy spores: item type (wallboard, ceiling tile, carpeting, upholstered furniture); moisture content of the autoclaved material (wet, dry); autoclave packing density (loose, dense); packing orientation (horizontal, vertical); opening autoclave bags prior to the cycle; autoclave temperature and pressure (31.5 lb/in² and 275°F, 45 lb/in² and 292°F); time in autoclave (up to 2 h); and multiple sequential autoclave cycles.

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

Item types. Unpainted wallboard (LaFarge regular grade 0.5-in.-thick drywall) was cut into sections that were approximately 2 ft by 2 ft. Sample BDR bags were

FIG. 3. Control placement (carpet and ceiling tile shown). TC, thermocouple.

TABLE 1. Test matrix

Run	Type of BDR ^a	Packing arrangement	Pressure (lb/in ²)	Temp (°F)	Time (min)
1	Mixed	Loose, horizontal	31.5	275	120
2	Wallboard	Dense, horizontal	45	292	120
3	Carpet	Loose, dense, large roll	31.5	275	120
4	Mixed (including sofa)	Loose, horizontal	45	292	75
5	Mixed	Loose, vertical	31.5	275	40 (two sequential runs)
6	Mixed	Loose, vertical, open bags	31.5	275	40 (two sequential runs)

^a Mixed material consisted of wallboard, ceiling tile, and carpet.

prepared by placing five of these sections face to face in autoclave bags. Wallboard was tested both wet and dry. In this context, dry refers to as-is condition at ambient humidity with no additional moisture added. Wet samples were submerged in a tank of water for 30 s and placed on a drain rack for 5 min before they were placed in the bag. Dry test bags weighed approximately 34 lb, and wet bags weighed approximately 37 lb. Samples were double bagged in 1.8-mil polypropylene autoclave bags (to represent likely practices that would be found during an emergency response), and the bags were individually goosenecked and taped shut using duct tape. A section of nylon rope was attached to the gooseneck to allow 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 “one-sample” bags) were assembled with one thermocouple and one test strip pouch placed together between the second and third wallboard sections. Other test bags (called “three-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 prepared without thermocouples and BIs and were used as fillers.

Ceiling tiles (Armstrong Contractor Series model 942 0.625-in.-thick ceiling panels) were cut into sections that were approximately 2 ft by 2 ft. Samples were prepared like the wallboard samples; however, the bags contained nine sections (2 ft by 2 ft) placed face to face. Dry test bags weighed approximately 23 lb, and wet bags weighed approximately 31 lb. “One-sample” bags contained one thermocouple and one test strip pouch placed together between the fourth and fifth ceiling tile sections. “Three-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 prepared without thermocouples and BI test pouches and were 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 that were 26 in. wide and 20 ft 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 like the wallboard and ceiling tiles. Dry test bags weighed approximately 26 lb, and wet bags weighed approximately 40 lb. As a worst-case model, larger sections of carpet that were 6 ft wide and 24 ft long were also tested. Only large rolls that were wet were prepared, and they weighed approximately 200 lb, the maximum size that could be reasonably handled by two workers. The large rolls were wrapped in polypropylene, and all seams were sealed with duct tape. For the small carpet rolls, “one-sample” and “three-sample” bags were prepared. “One-sample” bags contained one thermocouple and one test strip pouch placed together at the approximate midpoint of the radius of the carpet roll. “Three-sample” bags contained three thermocouples paired with three test strip pouches placed two laps from the top, at the midpoint of the radius, and two laps from the center of the carpet roll. Additional bags were prepared without thermocouples and BI test pouches and were used as fillers. The autoclave bins at the two ends of the group of bins used for each run were filled with BDR material without instruments to provide thermal mass and to minimize the impact of any cold spots within the autoclave.

To represent upholstered furniture, a dry, used, queen size sleeper sofa was autoclaved in run 4. Four thermocouples and four test strip pouches were paired and embedded at the following locations in the sofa: one sample each was inserted into holes cut approximately 6 in. deep in a back cushion and a seat cushion (the holes were then covered with duct tape); one sample was placed inside the folded sleeper mattress; and one sample 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 using two 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 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 in the load in the bin. Ceiling tiles were tested only using 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 min specified in the test plan. Carpet was tested in three configurations. Small rolls, approximately 1 ft in diameter and 26 in. 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 ft long and approximately 1.5 ft in diameter was tested in one run. Figure 4 shows the dense and loose packing arrangements.

FIG. 4. Dense and loose packing arrangements (wallboard shown).

FIG. 5. Vertical BDR positioning (wallboard and ceiling tile shown).

Packing orientation. Material was tested both lying horizontally (Fig. 4) in the autoclave bins and positioned vertically, with all sides exposed. Material was positioned vertically by tying two ropes to the top of a bag and attaching the ends of the ropes to opposite sides of the autoclave bin wall, as shown in Fig. 5. The bags were hung vertically to simulate a rack system and 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, these conditions would facilitate steam penetration and more effective heating of the material in the autoclave.

Open bags. All BDR was double bagged in 1.8-mil polypropylene autoclave bags. The bags were individually goosenecked and sealed with duct tape. This procedure was adopted based on packaging information from the State Department, Sterling, Va., mail facility anthrax cleanup (1). 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 bag 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 min at an elevated temperature (275°F), which is the standard operating conditions for the Healthcare Environmental autoclave. Previously published

FIG. 6. Time and temperature data (loose packing). TC, thermocouple; psig, lb/in².

FIG. 7. Temperature and wallboard spore viability for run 2. TC, thermocouple; psig, lb/in².

data indicate that holding material for 15 min at 250°F is required to ensure moist heat sterilization (2–4, 7). Therefore, the test plan called for extending the run time to more than 40 min so that the 250°F temperature target was reached at all, or at least most, embedded thermocouples. Even if the 250°F target had not been reached, the test plan established a maximum run time of 120 min to enable the autoclave to process multiple test runs each day. Runs 1, 2, and 3 were terminated at 120 min, before all the thermocouples reached the target temperature. Run 4 was stopped at 75 min because the sofa temperature was rising above the temperature of the autoclave, indicating that there was a potential exothermic reaction in the sofa. Because the 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-min 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 postvacuum cycle, from condensed water being drawn out of BDR under vacuum, or from 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 prevacuum, steam pressurization, and postvacuum phases. The cycles were conducted with no time between them, and the autoclave remained sealed throughout both cycles.

RESULTS

Figures 6, 7, 8, 9, 10, 11, and 12 show plots of the time and temperature data recorded during each of the six runs. Some of the figures (Fig. 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 and temperature.

Figure 6 shows the time and 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 (Fig. 1). After run 1, the bundle of thermocouple wire outside the flange was positioned so that gravity prevented condensate from collecting, and in subsequent runs there was only a minimal amount of noise. It should be noted that although the temperature of the control thermocouple rapidly approached the autoclave operating temperature, the temperature of many of the thermocouples never reached the target

FIG. 8. Effect of second autoclave cycle on reaching the target temperature (average measurements). psig, lb/in².

temperature, 250°F. The BI viability measurements obtained in run 1 were consistent with the temperature measurements (i.e., the BI strips that were at locations where 250°F was maintained for 15 min showed no growth).

Run 2 (Fig. 7) consisted of subjecting only densely packed wallboard to the higher autoclave pressure and temperature. Again, even at the higher temperature, the temperature of many of the thermocouples never reached 250°F. Wallboard is composed mostly of $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ and loses moisture at temperatures between 212 and 302°F (5). This dehydration step could have contributed to the slow heatup for wallboard, although the bulk density or packing density of the wallboard could also have been 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 time, which convinced the investigators that the problem was with the one thermocouple. In addition, the temperature sig-

FIG. 9. Effect of second autoclave cycle on spore survivability. psig, lb/in².

FIG. 10. Effect of second autoclave cycle with cut bags. TC, thermocouple; psig, lb/in².

nals converged at the end of the run. This observation led to the hypothesis that a second autoclave cycle might be effective.

The data shown in Fig. 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 observed in both the 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 population consisting of 10^6 spores. In a limited number of cases, the growth test indicated a positive result; however, the subsequent assay analysis revealed no quantifiable population (<100 CFU). These data series were labeled indeterminate. Note that for the sample locations at which the temperature was maintained at 250°F for 15 min the data consistently showed that there was no growth on the corresponding BI strips, while the data for most of the sample locations that did not meet these time and temperature targets showed that there was growth.

Figure 8 shows the effect of the second autoclave cycle (run 5), based on average temperature data for each bag. During this run, bags containing various materials were placed upright

FIG. 11. Effect of packing density for wallboard. psig, lb/in².

FIG. 12. Effect of moisture content. psig, lb/in².

to maximize exposure during the autoclave cycle, and then a second cycle was performed, 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. We believe that when the cold, porous BDR material was exposed to the steam during the first cycle, condensate formed in the pores, limiting steam penetration and subsequent heat transfer. With the pores of the material full of water, heat was transferred to the interior of the material mostly through conduction, which was slow, and the steam could not penetrate very well into the material. At the initiation of the second autoclave cycle, the evacuation step pulled the condensate out of the pores, so that when steam was reapplied, it effectively penetrated the preheated material and the temperature reached the operating temperature of the autoclave. The only thermocouples at which the necessary temperatures were not reached 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 data for run 5. As before, the samples that did not achieve the necessary time/temperature for decontamination exhibited residual spore viability.

Figure 10 shows the time and 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, the temperature of all thermocouples reached the required temperature for the time necessary for spore destruction, and this finding was 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 could be made about the effect of changing bag material as a means to promote bag burst during the autoclave cycle. However, these observations did suggest that packing BDR using bags made from a material that melts or opens during autoclaving might ensure good steam penetration.

Figure 11 shows the effect of packing density when wallboard was processed. Clearly, high-density packing reduced the effectiveness of the autoclaving. It appears that an auto-

clave facility processing BDR should minimize packing density so that steam can readily penetrate into each bag in the load.

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 more slowly than the other BDR types probably because the micropores of the ceiling tiles completely filled with water. The other item types showed similar heating profiles. This finding supports the hypothesis that initial condensation of steam in the pores of the ambient-temperature BDR limits heat transfer.

DISCUSSION

In this paper we describe an empirical study to evaluate whether moist heat and steam can successfully reach all surfaces of porous building materials and furnishings with sufficient potency 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 is important to acknowledge that weaponized spores, or even live nonweaponized spores, 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 with a stimulant, such as *G. stearothermophilus*. However, *G. stearothermophilus* is commonly used as a simulant for agents such as *B. anthracis*, particularly for studies of technologies utilizing thermal treatment methods to kill the spores (9). 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 contained 1×10^6 spores represented a worst-case scenario.

Based on the results of these tests, heating the BDR to 250°F for 15 min at the sampling locations resulted in no viable spores. The most effective spore destruction was obtained with a loose packing arrangement, dry BDR material, a higher autoclave operating pressure and higher temperature, multiple autoclave cycles performed in sequence, and bags cut open prior to loading.

The optimal practices for processing BDR in a commercial autoclave are as follows: place BDR so that all surfaces are exposed to the 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 when cut bags and two sequential autoclave cycles were used.

Our conclusions regarding autoclave operating conditions are as follows: treatment for 120 min at 31.5 lb/in² and 275°F decontaminated wallboard, ceiling tiles, and dry carpet when the materials were loaded as recommended; treatment for 75 min at 45 lb/in² and 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 result in spore destruction; treatment for 75 min at 45 lb/in² and 292°F decontaminated wallboard and ceiling tiles when the materials were loaded as recommended;

and two standard autoclave cycles consisting of 40 min at 31.5 lb/in² and 275°F in sequence decontaminated wallboard, ceiling tiles, and dry carpet when the materials were loaded as recommended. It may be possible to shorten the second cycle and still destroy the spores in the BDR; a third cycle may be necessary for wet carpet.

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

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