Final Study Report

USDA-APHIS Waste disposal project: Evaluation of a STI shredder and steamer disposal system for disposal of regulated garbage at USA ports

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Executive summary

A waste disposal study was conducted by the USDA-APHIS-PPQ-CPHST laboratory in Fort Collins, CO to determine the suitability of a shredder and steaming system for sterilizing bio-contaminated waste or quarantine materials. The shredder-steamer (STI Series 2000) is manufactured by Biosafe Engineering and has a waste capacity range of 300 to 6,000 lb/hr (136 to 2,722 kg/hr). This system could be used to sterilize contaminated soil or wood chips for any domestic emergency involving plant pests, or sterilize regulated garbage, and Quarantine Material Intercepted (QMI) waste to exclude pests from ports and border stations. The STI has a heavy duty shredder that can also shred confiscated electronics, packaged food, and clothing that may be seized at seaports. Also, the STI can be modified with optional waste hoppers and feeding augers to handle wood chips from orchard cleaning emergencies, or contaminated soil from a pathogen or insect infestation. The system was modified with sliding valve gates to regulate the steam temperature if the system was located at any elevation within the USA. The modified system was tested twice in 2012 and 2013 by the CPHST Fort Collins laboratory to determine its suitability for waste disposal for regulated garbage at USA ports, and for use in domestic plant emergencies to sterilize contaminated plant material, soil, or other contaminated materials.

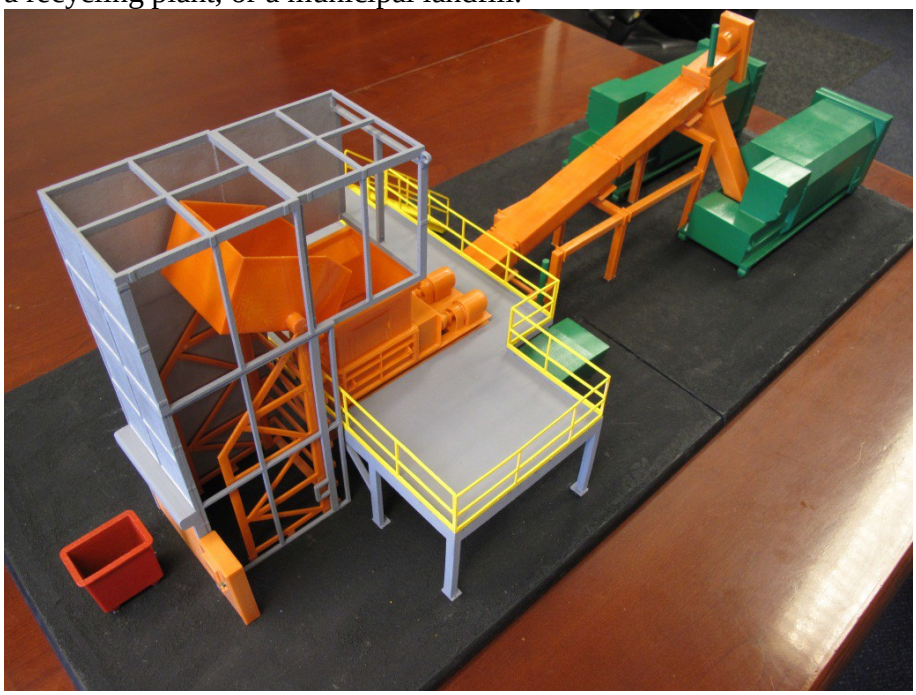
The first shredder steamer field study was conducted at the Biosafe Engineering facility in Brownsburg, IN in October, 2012. The STI system was tested with optional baffles added which regulated the steam pressure and the steam auger temperature. The STI system was evaluated for temperature dynamics and sporicidal efficacy for three replicated test runs. The total sample size for three runs was 240 samples, and each run had 80 samples, or 20 samples per treatment. The five treatments for the *Bacillus subtilis* efficacy test were: 1) untreated control samples, 2) uncut wool samples without any sandwich materials, 3) wool samples sandwiched in folded poster board and stapled around all the edges, 4) wool samples sandwiched in folded leather that is stapled around all three edges, and 5) cut wool samples without any sandwich materials. Two thermocouple sensor/data loggers were added to each test run to measure temperatures over time as the waste traveled up the auger inside the steam jacket. The sandwiched samples had no viable CFU spore counts for all 60 treated samples, i.e. there was 100% efficacy for these treatments. Also, two out of four STI treatments had 100% efficacy for *B. subtilis* spores. The autoclave had 100% *B. subtilis* spore efficacy for the 60 treated samples. These results indicate that the STI steaming system had an efficacy rating almost equivalent to the autoclave results. The USDA waste disposal standard for heat treatment is 212 F (100 C) for 30 minutes. There was an accidental pressure release problem in run #1, thus the temperature dropped slightly below 212 F (100 C) for approximately 10 minutes. Despite the accidental steam release the temperature was above 212 F (100 C) for approximately 35 minutes during the first run. The average temperature for runs 2 and 3 ranged from 217.4 to 221 F (103 to 105 C), as estimated when auger temperatures were above 212 F (100 C). The average time when the waste temperature was above 212 F (100 C) was 69 and 38 minutes for runs 2 and 3, respectively. These results show that the shredder and steaming technology, such as the STI Series 2000 system, can effectively decontaminate bio-contaminated waste, and therefore should be considered an acceptable heat treatment technology for regulated garbage waste disposal at USA ports and border stations.

The second STI field study was conducted at the Biosafe Engineering facility in Brownsburg, IN in March, 2013. The same modified STI system, as previously tested in Oct. 2012, was retested but with different *B. subtilis* samples for the sporicidal efficacy tests. Both replicated studies were conducted with a steaming system that had optional baffles added which regulated the steam pressure and the steam auger temperature. The STI system was tested for temperature dynamics and sporicidal efficacy for four test runs that included a total of 160 wool samples and 80 wood chip samples. The USDA waste disposal standard for heat treatment is 212 F (100 C) for 30 minutes. The temperature results show an average time of exposure and temperature of 57 min. and 217.4 F (103 C), respectively, across both sensors and all four runs. The temperature dynamic results show that the STI system met the USDA standards for waste disposal. The *B. subtilis* efficacy results for the wool fabric samples had an average log10 reduction of 5.68, across all four test runs. The average log10 reduction for the wood chip samples was 4.72 log10 CFU/wood chip, across all four test runs. The STI sporicidal efficacy results for the wool fabric samples show an > 6 and 5.68 log10 reduction for the Oct. 2012 and the Mar. 2013 field studies, respectively. Both the Oct. 2012 and the Mar. 2013 STI studies were conducted with the optional baffles, but the wool-nylon samples were prepared and treated under different conditions. A sporicidal efficacy study for medical waste treatment was conducted by an independent microbiology laboratory in 2004 on the STI system, but without the optional baffles. This study utilized *Bacillus atrophaeus* spore strips and had an average of > 6 log10 reduction for three separate tests runs at two different test sites. Including this independent study, the STI system has been evaluated in three separate sporicidal efficacy studies. These results show that the shredder and steaming technology, such as the STI Series 2000 system, can effectively sterilize bio-contaminated waste, and therefore should be considered an acceptable heat treatment technology for regulated garbage waste disposal at USA ports and border stations.

Introduction

The goal of this study was to evaluate a commercial shredder and steaming system to determine whether it could effectively sterilize regulated garbage or Quarantine Material Intercepted (QMI) bio-contaminated waste at USA ports and border stations (Photo 1). The same technology could be manufactured as a mobile system to decontaminate plant material, soil, confiscated materials, and other contaminated materials during a domestic emergency involving plant pathogens or plant insects. In September 2011, the CPHST Fort Collins Lab initiated a multi-year waste disposal project, which was funded by the USDA Farm Bill. The project's objective was to evaluate alternative methods to devitalize and dispose of agricultural waste (plant and animal materials) seized at U.S. ports of entry. In October, 2012 a shredder/steam waste disposal system, manufactured by Biosafe Engineering, LLC was tested for bacterial efficacy and for meeting USDA waste disposal standards. The USDA-APHIS treatment standard for waste disposal is 212 F (100 C) for 30 minutes. This test was part of a larger waste disposal project to compare the ability of four or five different sanitation systems to devitalize any seized agricultural products at USA border stations and air/sea ports. The Biosafe shredder/steamer (STI Series 2000) consists of a large shredder and an enclosed steam auger. The waste is first shredded into 7 mm particles and is then carried into the steam jacket using a twelve inch auger. Since 1995 Biosafe has sold 30 shredder/steamer systems to research labs, medical clinics/hospitals, and private waste disposal companies without any citations, or non-compliance complaints to date. The Biosafe waste disposal system was modified so that the steam auger could be pressurized to hold a specified temperature of 212° F (100 C) at any elevation within the USA.

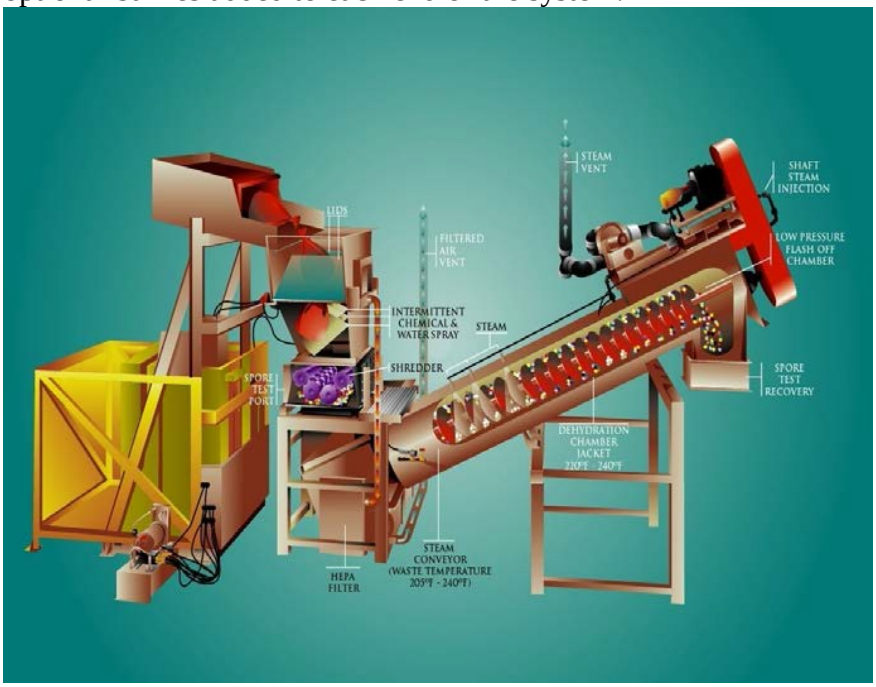
Photo 1: Scale model from 3D printer of STI shredder/steamer disposal system, as envisioned for a municipal waste facility. Note the automated loading system, and the two waste compactors at the right hand side of the model. The compactors can then be hauled to a recycling plant, or a municipal landfill.



The October, 2012 STI test used green wood chips as the waste carrier for the bacteria spore samples. This study was designed to test the STI for sporicidal efficacy. The test also included waste temperature measurements inside the auger. Wood chips were used to determine whether the STI system could effectively handle wood chips, for possible exportation to EU countries as a biofuel for electrical cogeneration. The STI efficacy tests were conducted to confirm that the system could meet the EU regulations for phytosanitary requirements to export wood chips to EU countries. The ISPM-15 standard for heat treatment of wood products for export is 133 F (56 C) for a minimum of 30 minutes for controlling wood boring insects. A final report for the 2012 study was written up and distributed within CPHST and sent to the PPQ regulated garbage directors.

A second STI study was conducted in March 2013 to collect more temperature data and re-confirm that the auger temperature matched or exceeded the USDA waste disposal standard for 212 F (100 C) for 30 minutes. A second objective of this project was to re-test the sporicidal efficacy of the STI system for *B. subtilis* spores that were dried onto both wool fabric samples and the surface of wood chips. The test was conducted at the Biosafe Life Science facility in Brownsburg, IN. The test was conducted with a STI Series 2000 model with a waste capacity of 600 lb (272.2 kg) per hour (Fig. 1). The STI system that was evaluated in this study was a commercial system that was modified with two slide gate pressure valves added at each end of the system so that the steam pressure could be regulated within the steam jacket, which in turn regulates the temperature within the auger cylinder. The shredder component of the STI system was not installed for this study, so that the wood chips would not be shredded and left as whole chips to evaluate the sporicidal efficacy section of the study.

Figure 1. Schematic of STI shredder and steaming waste disposal system, without the optional baffles added to each end of the system.



Sterile Technology Industries (STI) manufactures the STI Series 2000 shredder and steaming disposal system at the Biosafe Engineering, LLC facility in Brownsburg, IN. The first STI unit was sold in 1995, and over the past 19 years the STI systems have processed in excess of two billion pounds of biological waste at hospitals, medical clinics, medical waste disposal services, and for municipal waste companies. The STI systems range in waste capacities from 300 pounds (136.1 kg) per hour up to 3 metric tons per hour. STI systems have been approved for medical waste disposal in all 50 states in the USA. Dozens of commercial units have been placed in the United States, Ireland, Northern Ireland, Australia and England.

The STI system utilizes a combination of shredding and steam heat to decontaminate waste so that it can be safely disposed at municipal landfills. The heavy duty mechanical shredder reduces all waste down to about a 7 mm particle size. The smaller particle size allows rapid heat transfer into all of the waste, without leaving waste large enough to insulate pathogens from the heat transfer. Also, the shredder reduces the volume of waste by approximately 90%, depending on the waste composition. The waste is heat treated in the steam auger, which decontaminates any contaminated particles. The auger speed is computer controlled to regulate the time of exposure and ensure that all the shredded particles are effectively heated to deactivate the majority of pathogens usually found in medical waste. The STI is under negative air pressure and the H.E.P.A. filter system prevents the release of airborne pathogens during the integral shredding process. Built in sensors and data loggers monitor and record the temperature for each waste run. Steam is directly injected into the rotating auger to ensure rapid heating and complete mixing with the waste material.

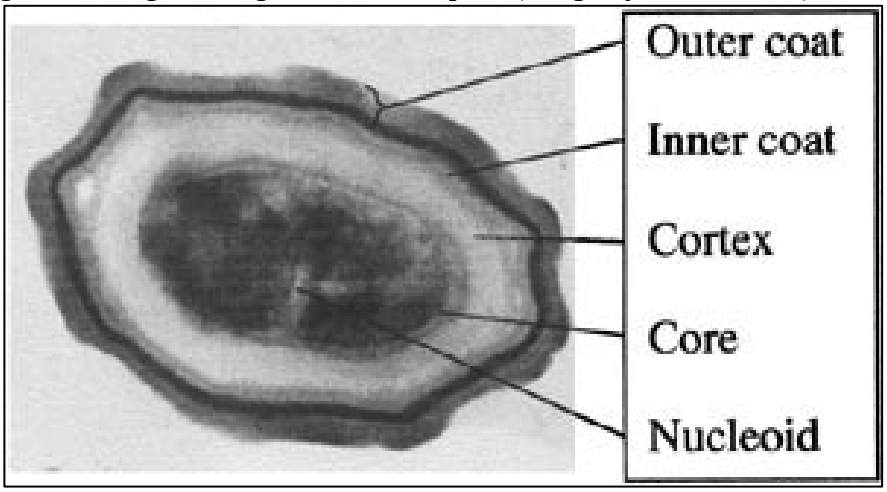
Background information on temperature effects on insect and bacterial spores

The efficacy of heat treatments on insects and insect eggs has not been fully summarized or reviewed, but several government standards have indicated the temperature and time requirements for controlling quarantine insects in wood products. The International Plant Protection Convention (IPPC) standard heat treatment for wood packaging material (WPM) for insects and pests is outlined in their International Standards For Phytosanitary Measures-15 (ISPM 15) manual. The ISPM 15 states that a minimum core temperature of 132.8 F (56 C) for 30 minutes is needed to control quarantine insects and pests in WPM. Unfortunately, there are many studies that do not support the use of the ISPM 15 treatment for wood handicrafts. In Meyers et al. (2009), a minimum core temperature of 140 F (60 C) for 60 minutes was necessary to kill *Agrilus planipennis*, the emerald ash borer (EAB), in firewood. Nzokou et al. (2008) showed a core temperature of 65 C for 30 minutes was necessary to kill EAB, and that EAB emergence was still observed after logs were heated to a core temperature of 140 F (60 C) for 30 minutes. As stated in Code of Federal Regulations (7CFR319.40-7(c)) "Heat treatment procedures may employ steam, hot water, kilns, exposure to microwave energy, or any other method (e.g., the water and steam techniques used in veneer production) that raises the temperature of the center of each treated regulated article to at least 160 F (71.1 C) and maintains the regulated article at that center temperature for at least 75 minutes." The use of commercial kiln wood-drying procedures could be used to kill quarantine pests and reduce the possibility of damage to wood handicrafts (7CFR319.40-7(d) (i)). The heat treatment outlined in 7CFR319.40- 7(c), which is 159.98 F (71.1 C) for 75 minutes, to kiln dry lumber and logs should effectively control pests in wood handicrafts. APHIS imposed a requirement that in order

for foreign lumber and logs to be imported into the United States, they must undergo heat treatment of 159.98 F (71.1 C) for 75 minutes – these tougher limits were felt to be justified in order "to protect U.S. forests from all pests, whereas the European and Korean regulation are targeted to more specific pest threats, such as the pinewood nematode" (Fantozzi, 1995). Besides dry heat, there are treatments that can be used for wood heat treatments. Heat treatment procedures may employ steam, hot water, kilns, exposure to microwave energy, heat/vacuum chambers or any other method (e.g., the water and steam techniques used in veneer production) that raises the temperature of the center of each treated regulated article to at least 159.98 F (71.1 ° C) for 75 minutes. There is a paucity of heat treatment research on wood pests. Additional research is necessary before the treatment temperature and duration can be lowered from 159.98 F (71.1° C) for 75 minutes. In general, the heat treatment needed to kill wood boring beetles is lower than the temperatures and time of exposure needed to kill spore forming bacteria. Thus, the heat treatment requirements for sterilization of bacterial spores will also encompass all the heating requirements to kill insect and insect eggs in waste that is shredded into small particles for rapid heat transfer across the entire particle.

A spore forming bacteria, *Bacillus subtilis* was chosen as the target microbe for the efficacy tests because it is a non-pathogenic bacterium that can be tested without any biosecurity containment methods, or ePermits. Also, the non-pathogenic spore can be safely handled during the study without any human health risks. A heat treatment study by Warth (1978) found that *B. subtilis* spores exposed to 230 F (110 C) for 32 minutes had 99% devitalization rates, or a 1% survival rate. The *B. subtilis* spores have a thick outer coating that makes the spores extremely difficult to devitalize with heat treatment or disinfectants (Fig. 2). According to the Spaulding classification (McDonnell and Burke 2011) that was originally proposed in 1957, bacterial spores were the most resistant microbe/pathogen to control with either heat or disinfectants. Since the

Figure 2. Image of single *B. subtilis* spore (image by: S. Pankratz)



Spaulding classification was developed two other microbe/pathogens have been added above the spore forming bacteria (Table 1). A heat treatment study by Warth (1978) found that *B. subtilis* spores exposed to 230 F (110 C) for 32 minutes had 99% devitalization rates, or a 1% survival rate. A 1% spore survival rate, based on an initial, baseline count of 10^7 spores, would

translate into 100,000 spores per ml or carrier that survived a heat treatment. Ultimately, the decision criteria for any waste disposal efficacy evaluation will be, "How clean is clean enough?" This will require a clear understanding of the microbial counts whether they are based on log10 reduction, or percent reductions. A review article by Rice et. al. (2005) shows that *Bacillus* species have differences in sporicidal efficacy that were dependent on both temperature and time of spore exposure to the heat. An independent microbiology laboratory test was conducted in 2004 by WNWN International Inc. to determine the sporicidal efficacy for the STI system. Two identical STI sporicidal efficacy tests were conducted at the Muhlenberg Medical Center and at LabCorp, which are both located in New Jersey. The two tests revealed that there was no growth of *B. atrophaeus* in any of the 18 spores test samples indicating a > 6 log10 reduction. These tests were conducted with an STI system without the optional baffles, and the results demonstrate the ability of the STI Series 2000 system to effectively treat medical waste. Research has shown that *Bacillus subtilis* spores have a

Table 1. Classification of microbial resistance to disinfectants and sterilants, based on McDonnell (2007). Micro-organisms are listed in order (highest to lowest) of known resistance to disinfectant inactivation, but this will vary depending on the disinfectant. It cannot be taken for granted that efficacy against micro-organisms with higher resistance will be effective against micro- organisms lower in the list.

Micro-Organism	Examples
Prions	Scrapie, CreutzfeldeJakob disease, chronic wasting disease
Bacterial spores	*Bacillus, Geobacillus, Clostridium*
Protozoal oocysts	*Cryptosporidium*
Helminth eggs	*Ascaris, Enterobius*
Mycobacteria	*Mycobacterium tuberculosis, M. terrae, M. chelonae*
Small, non-enveloped viruses	Poliovirus, parvoviruses, papilloma viruses
Protozoal cysts	*Giardia, Acanthamoeba*
Fungal spores	*Aspergillus, Penicillium*
Gram-negative bacteria	*Pseudomonas, Providencia, Escherichia*
Vegetative fungi and algae	*Aspergillus, Trichophyton, Candida, Chlamydomonas*
Vegetative helminths and protozoa	*Ascaris, Cryptosporidium, Giardia*
Large, non-enveloped viruses	Adenoviruses, rotaviruses
Gram-positive bacteria	*Staphylococcus, Streptococcus, Enterococcus*
Enveloped viruses	Human immunodeficiency virus, hepatitis B virus, herpes simplex virus

germination rate of 18 to 68% after 15 to 120 minutes of incubation, following an 81 day storage at 39.2 F (4 C) (Powell, 1950). These results suggest that Bacillus spores can rapidly germinate from their spore stage back into their vegetative cells after removal from refrigeration, depending on the "incubation" temperature. If a disposal system efficacy test was conducted with *Bacillus* spores that reverted into vegetative cells, the results would probably

be biased towards a very high deactivation/efficacy rate. In this study, the *B. subtilis* samples were shipped in insulated containers with ice packs and stored at 39.2 F (4 C), up until 30 minutes before the samples were placed into the STI for each of the four test runs.

Materials and methods:

October 2012 Study design

The study was replicated three times for a sporicidal efficacy test. The first test used un-shredded, hay material. The second and third tests were run with fresh wood chips, collected from a local tree removal company. Each of the three test runs had 80 *B. subtilis* samples. Each test run consisted of four treatments, excluding treatment #1 which is the untreated control. The five treatments are described below (Table 2). There were a total of 320 *B. subtilis* samples prepared for this study. In addition there were 40 fabric samples for an autoclave test. The control samples (trt # 1) were left untreated, but were shipped and stored along with the other treatments in order to determine any effects of shipping and storage on the final *B. subtilis* CFU counts. The *B. subtilis* were embedded inside of wool fabric samples at a private laboratory, and then prepared into the samples described in Table 2. The five treatments for the *B. subtilis* efficacy test were: 1) untreated control samples, 2) wool samples without any sandwich materials, 3) wool samples sandwiched in folded poster board and stapled around all the edges, and 4) wool samples sandwiched in folded leather that is stapled around all three edges, Photo 2) , and 5) cut wool samples. The two *B. subtilis* sandwiches included an inoculated piece of wool/rayon fabric approximately 2 in. wide X 3 in. long (50.8 x 76.2 mm). Sandwiched samples were not shredded before being placed in the waste bin port hole for steam treatment. The fabric was inoculated with *B. subtilis* and dried for at least 24 hours under refrigeration. For two treatments the fabric was sandwiched in between two pieces of leather or poster board that are approximately 4 in. long x 3 in. wide (101.6 x 76.2mm). The two pieces of leather, or poster board, were stapled together to secure the fabric inside. Some samples in each of the three runs were cut into pieces to simulate shredding (treatment 5), and some were left uncut to test the effects of steam alone on *B. subtilis* efficacy (treatments 2, 3, and 4). The cut samples were not sandwiched with either poster board or leather, but left uncovered for this study. Another 40 wool samples were treated in the CPHST Fort Collins laboratory using the lab autoclave to sanitize the fabric samples that are not sandwiched by any material. The autoclave target temperature and pressure was set to 121 C (250 F) and 15 PSI for 30 minutes. The STI system operates at or above 100 C (212 F) at 2 to 5 PSI. The STI has a variable auger speed, but for this study the speed was set so the chips would be in the auger for approximately 40 - 60 minutes. The wool fabric sandwiched between the poster and leather were prepared in this manner to mimic waste conditions where spores may be embedded into woody or animal tissue and the waste bypassed the shredder by accident.

Bacillus subtilis preparation:

The spore samples were prepared at MicroChem lab in Euless, TX. *B. subtilis* was treated with isopropanol to kill the vegetative bacteria cells so that only the spores were alive when the liquid suspension was embedded onto the wool strips. The wool fabric was dried and refrigerated at 4 C to keep the bacteria in the spore stage. The baseline count for *B. subtilis* was approximately 10^8 CFU spores/ml. The samples were shipped overnight to the Biosafe facility in boxes with ice packs to reduce losses in CFU counts due to storage at room temperature.

Table 2. Description of treatments and sample size for the Oct. 2012 STI study

Treatment number	Treatment description	Sample size per run
1	Untreated fabric –preloaded with *B. subtilis*	40- total control sample size
2	Wool fabric alone	20
3	Wool fabric inside poster board sandwich sample	20
4	Wool fabric inside leather sandwich sample	20
5	Cut samples but treated along with sandwich samples	20
Total sample size per run		80

Photo 2. Wool/nylon samples embedded with *B. subtilis* spores are shown below fabric (lower left), poster board (upper left), and leather (right). The *B. subtilis* samples were not shredded before being steam treated.

STI steamer methods

The field study was conducted at the Biosafe Engineering facility where a modified STI shedder/steamer was available for testing. The shredder component of the system was not attached to the auger steamer for this study, so the treatment samples were placed in the auger bin without any shredding. If the shredder was present it could be bypassed by placing the test samples in a side port that feeds into the hopper below the shredder that feeds the steam auger. The two slide valve gates are controlled by a computer, and the gates allow the STI auger to hold a selected temperature of 212 F (100 C) when operating at any elevation above sea level. The pressure inside the steam treatment auger can be varied from 2 to 5 PSI to maintain the auger temperature at 212 F (100 C) at any expected atmospheric pressure.

For the first run one hay bale was partly used to add the samples to be treated. The second and third runs used wood chips. The inoculated samples were placed inside a plastic container with dozens of air holes so that the samples can be easily retrieved from the treated hay. The hay was steam treated along with all the treatment samples, so that the auger would be full. A full auger would absorb the most heat from the steam jacket, and simulates real waste disposal conditions.

Two MadgeTech thermocouple sensors/data loggers, made of stainless steel, were added to the hay or wood chips by a side port. The data loggers (Model -Temp 1000S) recorded the temperature in the auger at 15 second intervals. The temperature was recorded before entering the auger and after to determine the length of time any portion of the hay was in the auger and exposed to the steam temperature. Data loggers were started before entering the auger and stopped after the auger, so that the actual time in the auger can be estimated by temperature changes in the data.

The *B. subtilis* samples were collected and placed in pre-sterilized vials using sterile techniques at the end of the test run. Vials were stored in an ice chest with freezer blocks. The samples were mailed over night to the CPHST Fort Collins lab and stored in a freezer. All samples were collected, stored at the Fort Collins lab, and then sent to MicroChem lab. The samples were analyzed by MicroChem lab using their standard methods for washing spores off the wool fabric and then culturing the wash off suspension on media plates. MicroChem quantified the viable *B. subtilis* CFU counts in an Excel spreadsheet and wrote up a full description of their methods and analysis in their final report. They delivered a final report for their sample preparation, assay methods, and results for *B. subtilis* efficacy in Nov 2012. The final report was distributed to the associate directors in CPHST.

Wood chip moisture content was measured before and after steam treatment, with a balance at the CPHST lab. The chip size ranged from: 1” x 1” x 1/8” (25.4 x 25.4 x 3.175 mm) to 2 1/4” x 2 1/4” x 1/4” (57 x 57 x 6.35mm). Fresh wood chips (24 chips) were collected from the pretreated bin, placed in labeled vials, and capped to prevent moisture loss during shipping. Steam treated wood chips were collected at the end of the treatment cycle, placed in a labeled vial, and shipped back to the CPHST lab. The chips were weighed in order to estimate the moisture content relative to the initial chip moisture. The sample chips were then oven dried to constant moisture and then re-weighed to determine the treated chip moisture content relative to completely dry chip weights.

Autoclave test

The purpose of this test was to compare a standard autoclave steam treatment with the STI test results. For this test 60 wool samples were prepared by MicroChem with the same methods described in the STI test. The wool samples were the same size (2" x 3") (50.8 x 76.2 mm) and were not sandwiched with any material. There were three autoclave runs, using the steam autoclave unit at the CPHST Fort Collins laboratory. Twenty, uncut samples were tested for each of the three runs. The wool samples were placed in a 50 ml glass vial, then placed in the autoclave for treatment. The recorded autoclave temperature, pressure and time for this test were: 258 F (125.6 C)), approximately 18 PSI, and for 30 minutes. The samples were collected, and placed in labeled zip lock bags, and frozen until the shipping date. The untreated, white wool samples used in the STI test were also the control samples for the autoclave study. The control samples and the autoclaved samples were sent to MicroChem labs for analysis.

March 2013 Study design

The study is a completely randomized design with four replicate waste runs. Wood chips from a local tree trimming company were used as the waste in this test. The data collected for this study included temperature measurements in the wood chip waste, and *Bacillus subtilis* spore survival data for the four test runs. The study objectives were, 1) quantify the wood chip temperature inside the steam auger for each of the four runs, using two data loggers with temperature sensors, and 2) quantify the microbial efficacy of the STI steamer system after spiking wool fabric and wood chips with *Bacillus subtilis* spores and treating with steam heat >212 F (>100 C) for a minimum of 40 minutes of heat exposure.

Bacillus subtilis sample preparation at MicroChem laboratory:

Bacillus subtilis was cultured on media plates, and then scrapped off into a saline solution to form a liquid suspension to inoculate the wool samples and wood chips. Spore density was about 7 or 8 log 10 CFU/ml in the initial, liquid suspension, after an isopropanol treatment to kill off all the vegetative cells so that only spores remain. The samples were kept in the spore stage at the Biosafe facility, using refrigeration, until the day of testing. Each wool/nylon fabric sample was approximately 2" wide x 3" (50.8 mm wide X 76.2 mm) and had *B. subtilis* spores embedded and dried onto all of the thread surfaces. There were a total of 200 *B. subtilis* wool samples prepared for this study. Each of the four test runs had 40 samples, for a total of 160 treated wool samples (4 runs x 40 samples = 160 treated samples). In addition, there were 40 untreated wool control samples. The *B. subtilis* samples were shipped overnight in insulated packs to the Biosafe facility to reduce spore losses. The treated samples were shipped overnight in a freezer packs back to MicroChem lab for culturing and analysis.

The wood chips were sterilized in an autoclave before inoculating with the *B. subtilis* spores. The chips were then soaked in a culture of *B. subtilis* spores for approximately 10 minutes, and then removed and placed into a sterile container to dry for a minimum of three days. The inoculated and dried wood chips were placed into a sterile bag and shipped to the Biosafe facility for testing each of the four test runs had 20 wood chip samples, for a total of 80 treated wood chip samples. In addition, there were 20 untreated control wood chip samples.

The viable spore counts were measured at the MicroChem lab by placing the wood chips into a flask containing 20 ml of recovery medium and mixed on a vortex mixer for 1.0 minute to remove any surviving spores. One ten-fold dilution may be made as 1.0 ml into 9 ml recovery

media and 0.5 ml portions of the bottle and dilution was placed onto nutrient agar in petri plates and incubated. Serial ten-fold dilutions were made of the untreated controls and 0.5 ml of various dilutions were placed onto nutrient auger in petri plates and incubated. Colonies of *B. subtilis* were then counted and multiplied by appropriate dilution factors to determine the number of *B. subtilis* spores remaining on a wood chip.

STI steamer methods:
All the tests were conducted at the Biosafe facility with their modified STI Series 2000 with the pressure regulating slide gate valve system. The shredder component of the system was not attached to the auger steamer for this study, so the wood chips were placed in the auger bin without any further shredding (Photo 3). The patented baffle system allows the STI auger to hold a selected temperature when operating at any elevation above sea level. The pressure inside the steam treatment auger can be varied from 2 to 5 PSI to maintain the auger temperature at 212 F (100 C) at any expected atmospheric pressure. Two sets of slide gate valves are placed at each end of the system to effectively contain the steam pressure, via computer controls. The pressure was regulated so that the temperature was above 212 F (100 C), and the auger speed was set for a minimum wood chip exposure time of 40 minutes for each run.

Photo 3. STI shredder blades reduce waste down to an average size of 7 mm. The shredder size is matched to the STI capacity. The larger shredders are designed to shred large animal bones, and even confiscated computer hardware components.

STI auger temperature measurements:
The temperature inside the steam auger was measured at 10-second intervals with two temperature data loggers (Photo 4). The data loggers are made of stainless steel, with a temperature range of -40 to 302 F (-40 to +150 C), and a sensitivity of ±32.9 F (± 0.5 C). The data loggers were added to the wood chips by the port in the auger bin. The temperature was recorded before entering the auger and after exiting the auger to determine the length of time the wood chips were in the auger and exposed to the steam temperature. The temperature data recorded whether the wood chips reached 212 F (100 C) for 30 minutes as required by APHIS

waste disposal regulations. The data points were plotted over time in a graph to indicate the maximum temperatures during the time of the sensors traveling up the twenty six-foot (8 m) auger. There were eight sets of temperature data for the four runs.

Photo 4. Two temperature sensors and data loggers (MadgeTech Inc), used to collect data from the wood chip waste as it traveled up the STI auger.

B. subtilis sample preparation, handling and storage

Two different sporicidal efficacy tests were conducted together during each of the four replicate runs with the STI system. The two sporicidal tests used *B. subtilis* spores dried onto; 1) wool/nylon fabric samples, and 2) the surface of wood chips made for smoking chips for grilling which have flat and wide surfaces which are easy surfaces to use in this test. The three treatments were: 1) control, 2) wool samples, and 3) wood chip samples (Table 3). Each of the four test runs had 40 *B. subtilis* wool/nylon samples and 20 inoculated wood chips samples. The wood chip samples were placed in small, perforated PVC tubes with screw caps on each end. The PVC tubes were mixed in with the carrier wood chips that were purchased from a local tree trimming company. The tubes were then collected at the end of the auger, and the wood chips were split with a sterile knife blade and inserted in a pre-sterilized vial.

Table 3. *B. subtilis* sample numbers for each of four replicated test runs.

Bacillus subtilis samples	Treated sample number per run	Control samples per run	Total sample numbers for 4 runs
1. Control	na	15	60
2. Wool/nylon fabric	40	na	160
3. Wood chip surface	20	na	80
Total sample number			300

The wool/nylon *B. subtilis* spore samples were placed in perforated, stainless steel tubes for each testing run. The tubes are 3" (76.2 mm) long with an inside diameter of 0.5" (12.7mm) (Photo 5). The tube had one sealed end and one open end. The steel tubes were collected by hand from the treated wood chips. The wool samples were removed from the steel tubes with tweezers, and then placed in a sterile plastic tube and labeled by run, treatment, and vial number.

The wool/nylon test had a total of 40 control or untreated samples, to compare to the four sets of treated samples. The wood chip samples had a total of 20 control chips to compare to the four sets of treated wood chips. The total number of wool samples was 200 (4 x 40 = 160 treated samples + 40 control samples). The total number of wood chips was 100 (4 x 20 = 80 treated chips + 20 control chips). Control samples were shipped and stored along with the three other treatments in order to determine any effects of shipping and storage on the final *B. subtilis* CFU counts. Plastic eggs with both types of samples were placed in the bottom hopper

Photo 5. The wool/rayon samples embedded with *B. subtilis* spores were inserted into the perforated steel tubes on the right side of the zip lock bag.

of the STI unit, along with the bulk wood chips. Treated samples were collected from the waste, placed in labelled plastic bags and frozen within 15 minutes after the samples were collected from the waste.

The frozen wool and wood chip samples were shipped overnight in insulted boxes with ice packs to the MicroChem lab for culturing of viable *B. subtilis* spores. MicroChem lab cultured all samples on media plates, and analyzed any bacterial growth for viable CFU counts for *B. subtilis*. Under field study conditions there were many times and possibilities for the samples to be contaminated with other microbes. Sample contamination was minimized by using semi-selective media to grow out the *B. subtilis* spores. Also, MicroChem maintained an original culture from the sample preparation stage. Those spores were cultured on Petri plates during the sample assay to visually confirm that the sample spore growth characteristics were identical to the original spore growth characteristics. The sample cultures were visually identical to the

original cultures. MicroChem wrote a final report for their assay methods and results for *B. subtilis* efficacy tests. This report is available to anyone within PPQ or CPHST who is interested in waste disposal and technology testing methods.

Photo 6. STI steam system modified with slide valve gates, operating during March 2013 field test. Randall McKee, who is the inventor for the STI shredder/steamer system, is operating the STI for this study.

Study Results:

Oct. 2012 study results

The *B. subtilis* efficacy results were written up by MicroChem laboratory as a final report, and sent to the Fort Collins, CPHST lab. The results are for non-shredded samples, with two treatments with the wool samples sandwiched in leather or poster board. The efficacy results could be even higher if the samples were shredded before being steam treated. The efficacy results show a virtually complete devitalization of the *B. subtilis* spores embedded on the wool samples (Table 4). The viable spore counts are recorded as Colony Forming Units per sample, or CFU/sample. Viable CFU counts were averaged across all three runs. Also, the log10 reduction and percent reduction was calculated for each treatment.

The results show an average of l.92 x 10^8 viable CFU/sample for the control (treatment 1)**.** An average of 86 CFU viable *B. subtilis* spores per sample was recovered from the treated wool (treatment 2), and 64 CFU/sample were recovered from treatment 5. There were no surviving *B. subtilis* spores recovered for treatment 3 (wrapped in poster board), or treatment 4 (wrapped in leather), and for the autoclaved samples (treatment 6). The data analysis shows that the viable CFU counts for *B. subtilis* spores were reduced by > 6 log10 for all non-shredded, treated samples. The sandwiched samples had no viable CFU spore counts for all 60 treated samples, i.e. there was 100% efficacy for these treatments. The autoclave also had 100% *B. subtilis* spore efficacy for the 60 treated samples. These results show that two out of four STI treatments had 100% efficacy, which corresponds to the autoclave results for 100% efficacy of the spores. The STI temperature and pressure was 217.4 F (103 C) and 5 PSI, and

the autoclave had a temperature and pressure of 249.8 F (121 C) and 18 PSI. The STI system had comparable efficacy results with the autoclave despite the sample preparation differences and the lower temperature and pressure for the STI tests. The sandwiched samples had 100% efficacy, which indicates that the rate of heat transfer into the sample and the time of exposure was sufficient to kill all the spores.

Table 4. Teatment descriptions, total number of samples for three runs, viable *B. subtilis* CFU's/sample, log 10 reduction, and percent reduction for the Oct. 2012 study.

Treatment description	Total number of samples for 3 runs	Viable spores (CFU/sample)	Log10 reduction	Percent reduction
Untreated fabric-preloaded with *B. subtilis*	60	191,876,667	0	0
Wool fabric alone	60	86	6.36597	99.99998
Wool fabric inside poster board sandwich sample (plant waste)	30	0	Complete control	100
Wool fabric inside leather sandwich sample (animal waste)	30	0	Complete control	100
Cut samples (shredded) but treated along with sandwich samples	80	64	6.47266	99.999967
Autoclave	60	0	Complete control	100

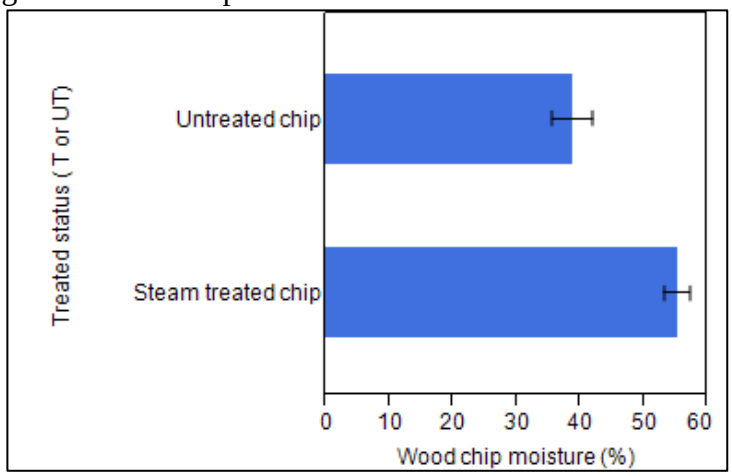
During the Oct. 2012 study the *B. subtilis* sample culturing process at the MicroChem laboratory was complicated by sample contamination during collection at the Biosafe facility. The samples were contaminated with other Bacillus species, with similar morphology and appearance on the media plates. Thus, there may have been some bacterial colonies that were misidentified, due to similar appearances. An original *B. subtilis* culture was then grown out in order to accurately compare spore growth for the poster board and leather treatments. The samples for treatments 2 and 5 that were not covered were counted before the original spores were cultured. Thus for treatments 2 and 5 the viable CFU counts could have been miscounted, due to inaccurate identification of another Bacillus species. However, the viable CFU counts for treatments 2 and 5 were still relatively low. The March 2013 study corrected for this misidentification among related Bacillus species, by saving the original sample preparation culture. During the assay of the treated samples the original culture was also plated out for visual comparison to correctly identify the spore growth on all the samples as *B. subtilis*.

Wood chip moisture content

The fresh cut, wood chip moisture was 39 and 55% for the fresh cut untreated and treated chips, respectively (Fig. 3). The treated chips had more moisture due to the steam treatment.

The steam heated wood chips may eventually have less moisture if allowed to dry completely. The steam heat may convert the initial moisture into a water vapor that is evaporated over time. Fresh wood chips have a moisture content of approximately 50%, and have a small heating value. If the moisture content could be reduced below 25%, then wood chip "self heating" and composting can be avoided. The energy density of wood chips can vary from 14.3 GJ/tonne at 25% moisture to 9.5 GJ/tonne at 50% moisture, indicating that chip moisture is a critical factor in economic evaluations for electrical cogeneration.

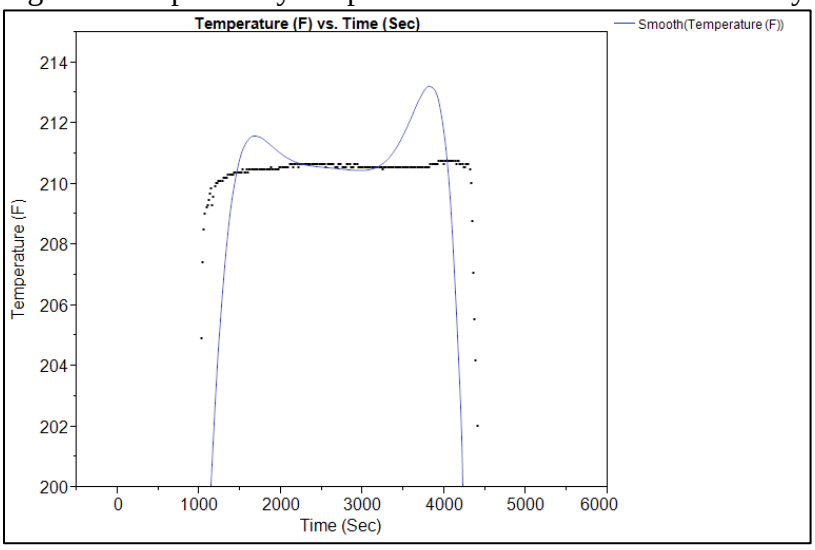
Figure 3. Wood chip moisture before and after steam treatment at 100 C for 35 min.



Preliminary STI test in 2011

In Sept. 2011 a preliminary STI auger temperature test was conducted with a hospital system that had been in operation at a hospital since May 2005. The temperature sensors were placed in the side port of the STI along with the *B. subtilis* bag samples. The sensors collected the

Figure 4. Graph of hay temperature inside a commercial STI system during the 2011 test.



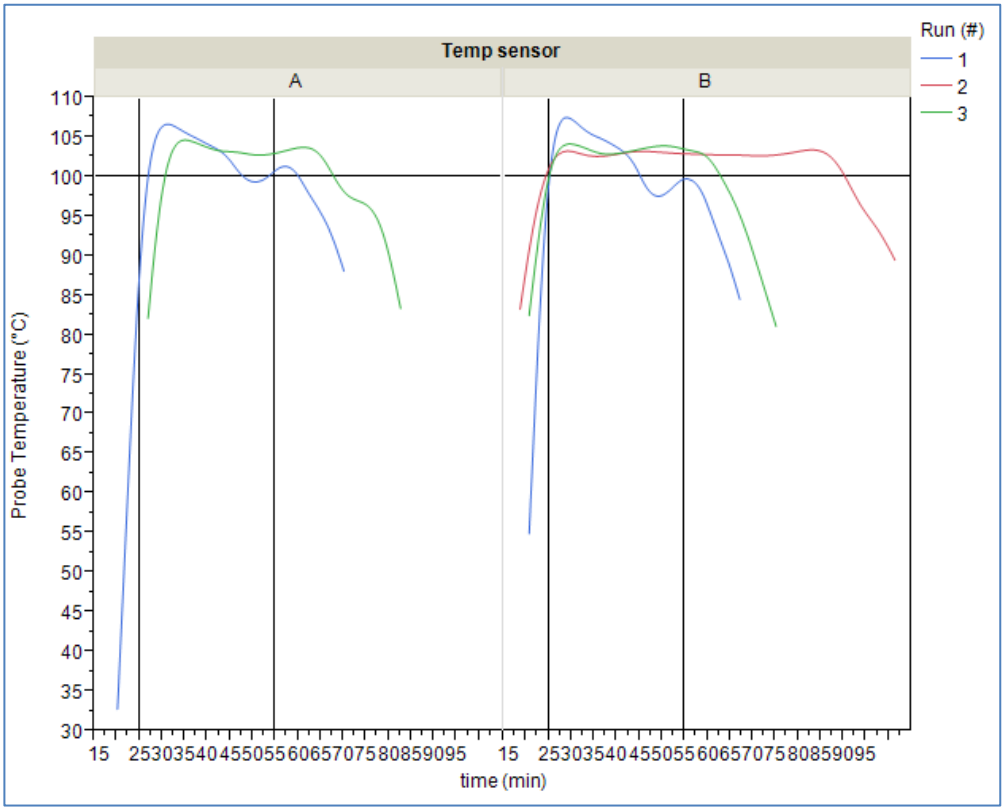
temperature of the hay at 15 sec intervals over a 1.5 hour time period. The average temperature of the hay inside the auger was 210.5 F (99 C), for approximately 50 minutes

when the sensor was in the auger (Fig. 4). During the first run, the temperature was above 212 F (100 C) for an average of 35 minutes.

Steam auger temperature dynamics for Oct. 2012 study

The auger in the STI is surrounded by a steam jacket, which delivers steam into the shredded waste or wood chips during its travel up the auger. The auger's speed is about 1 RPM, and is computer controlled to regulate the exposure time to the steam heat. The waste temperature dynamics were plotted as temperature over time for the two temperature data loggers (Fig. 5). The two plots show the measured temperature of the hay and wood chips inside the STI steam auger for each of the three runs. Temperature data were plotted for run one (blue line), run two (red line) and run three (green line), which show that the maximum temperatures were reached at about 25 to 35 minutes into each run. The two black, vertical lines are reference time lines that start at 25 minutes and end at 55 minutes, which is the 30 min. minimum required time for USDA waste disposal standards. The top, horizontal black line is the reference temperature line for 212 F (100 C), which is the minimum required temperature for USDA waste disposal standards.

Figure 5. Graph of hay and wood chip temperatures in steam jacket auger over time, for two Madge temperature sensors, for three STI runs in the Oct. 2012 study.



The two sensors were labeled A and B on the top x-axis, and the three test runs are labelled by three colored lines in the upper right legend. Temperature (C) is on the y-axis, and time (min.) is on the bottom x-axis. The average temperatures and exposure time, when the waste temperature was above 212 F (100 C), was estimated from the data (Table 5).

Table 5. Average temperature and exposure times for two temperature data loggers across three test runs during the Oct. 2012 STI study. Sensor A malfunctioned in second test run.

	Sensor A		Sensor B	
Test run	Ave. temperature	Number of minutes over 212F (100 C)	Ave. temperature	Number of minutes over 212F (100 C)
1	104	37	104	37
2	NA	NA	103	71
3	103	40	103	39
Ave. temperature or time	103	38	103	49

The USDA waste disposal regulations require a time and temperature of 212 F (100 C) for 30 minutes. The temperature dynamics graph delineates how long the wood chips were heated to a minimum of 212 F (100 C), while in the steam auger. There was an accidental pressure release in run 1, as recorded by both sensors. Thus the temperature dropped slightly below 212 F (100 C) for run 1 for approximately 10 minutes, but was above 212 F (100 C) for approximately 35 minutes during the first run. The temperature for runs 2 and 3 ranged from 217 to 221 F (103 to 105 C), during the time period when temperatures were above 100 C.

The results from this study also show that the steam treatment alone had 100% efficacy for *B. subtilis* spores embedded in wood fiber (poster board) or leather, without any pre-shredding of the material. This modified STI system used in this study did not have a shredder attached to the system. The complete STI system, sold commercially, has a shredder mounted before the steam auger which shreds all the waste down to an approximate size of 7 mm. The shredded waste has a much larger surface area, and faster heat transfer across each waste particle compared to un-shredded waste. The shredded waste should have no cold spots, or insulated spots that would lower exposure to the steam heat. The commercial STI shredder/steamer system should have an even more consistent, high efficacy rating as compared to the results from this test.

March 2013 study results

STI temperature dynamics results

The temperature dynamic results from this study can be visualized by graphing the temperature for each sensor, for all four runs, over time. Data were collected at 10 sec time intervals. A smoother line was generated from the data to plot the temperature dynamics over time, for each of the four test runs for sensor A (Fig. 6) and sensor B (Fig. 7).

Figure 6. Average temperature (C), graphed over time, for each of the four STI runs for sensor A. The color coded label for the four tests is in the upper right legend.

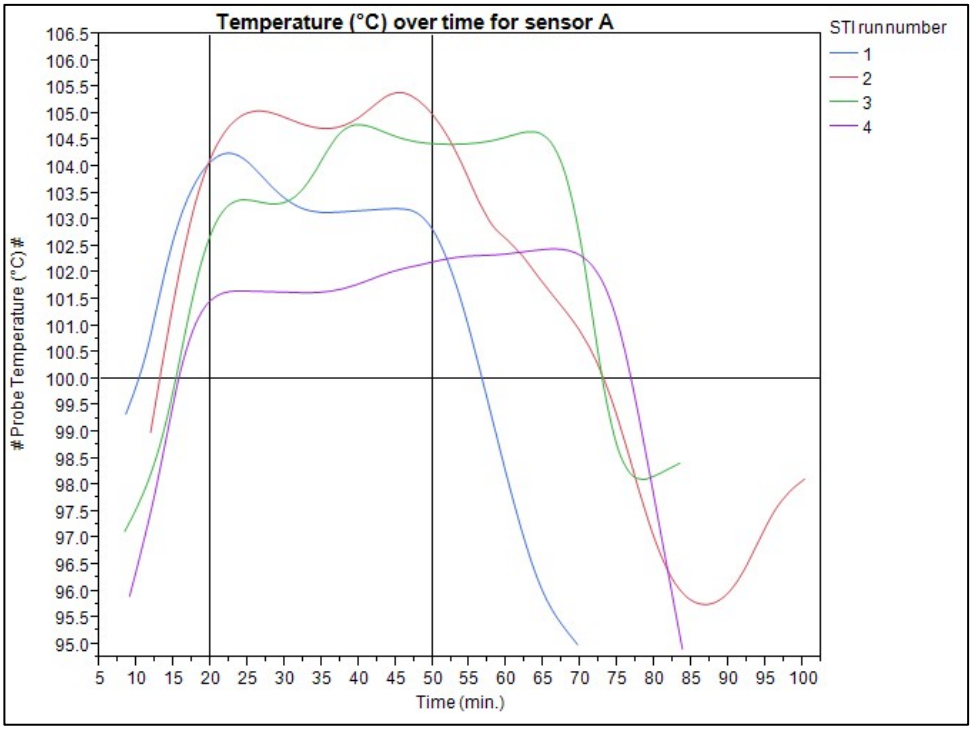
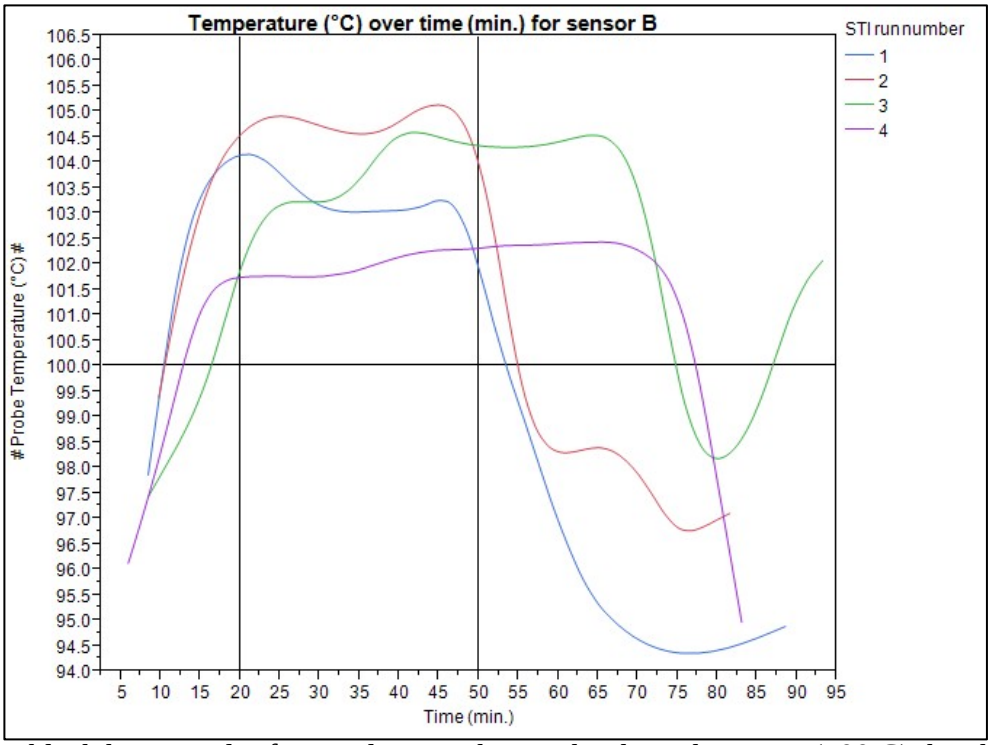


Figure 7. Average temperature (C), graphed over time, for each of the four STI runs for sensor B. The color coded label for the four tests is in the upper right legend.



The black horizontal reference lines in the graphs show the 212 F (100 C) threshold. All the colored lines are above 212 F (100 C), when referenced to the two vertical reference lines. The two vertical reference lines in the graphs represent the 30 minutes required by the USDA

waste disposal regulations. As can be seen in both graphs the wood chip temperatures were over 212 F (100 C) for about 40 to 60 minutes, for all four tests. The STI system is computer controlled so the auger speed and waste exposure time can be set for a wide range, depending on waste conditions or pest control requirements. For this set of tests the prototype STI system was not fully computerized, thus the steam jacket pressure was regulated manually, which resulted in temperature differences between the four runs. The two graphs show that the STI system met the USDA waste disposal requirements for 212 F (100 C) at 30 min.

The average time of exposure and average temperature was 57 min. and 217.4 F (103 C), respectively, across both sensors and all four runs (Table 6). Time of exposure was calculated from the data by subtracting the time differences (end time – start time), when the temperatures were equal to or greater than 212 F (100 C). Both sensors were placed together in a plastic egg for each run, so the wood chip exposure time should have been almost identical for both sensors. Any differences in temperatures between the sensors may be attributed to how the sensors were placed in the plastic egg, how the egg traveled up the auger in the wood chips, and eventually how the egg landed in the wood chip pile at the end of each test.

Table 6. Average wood chip time of exposure and average temperature for both temperature sensors for four test runs.

	Sensor A		Sensor B	
Run number	Ave. Temperature	Number of minutes over 212F (100 C)	Ave. Temperature	Number of minutes over 212F (100 C)
1	103	50	103	46
2	104	61	104	43
3	104	56	104	75
4	102	62	102	64

Photo 7. Wood chips embedded with *B. subtilis* spores were placed in the perforated plastic tube with capped ends, and then placed in the STI for steam treatment.

All of the commercial STI systems are fully computerized with built in, temperature sensors placed along the steam jacket and auger. A data logger in the STI system continuously monitors the temperature for each of the waste runs for quality assurance reporting. The current STI models range in capacity from 300 lb (136 kg) per hour up to 6,000 lb (2,722 kg) per hour. There are currently 33 STI units operating at medical clinics and hospitals, as well as at municipal waste facilities. The patented, slide valve gate system is an optional component that can be added to ensure that the STI temperature can be maintained at 212 F (100 C) for 30 minutes when placed at any elevation, such as the Denver International Airport.

Sporicidal efficacy results

The wool samples were not shredded, but rolled up and fitted into the stainless steel tubes. Wool fabric enclosed in a perforated steel tube probably does not allow the same heat transfer rate into the center of each rolled up sample as a small shredded particle.

Sporicidal efficacy for shredded and non-shredded waste was not a factor in this test, but the results may be dependent on how the samples were prepared for the steam treatment. The results for the *B. subtilis* wool fabric test show an average log10 reduction of 5.68, across all four test runs (Table 7). Log10 reduction is defined as the difference between the recovered CFU counts (log10 basis) for the control samples and the viable CFU counts in the treated samples. For example, if the initial spore count is 1,000,000 CFUs at the start of a test, then a 1 log10 reduction would reduce the viable CFU's down to 100,000 or 90%. A 6 log10 reduction would reduce 1,000,000 CFU's down to 1 CFU which represents a 99.9999% reduction in viable CFU counts. The percent spore recovery from the samples could not be

Table 7. The average *Bacillus subtilis* log 10 reduction and percent reduction for wool fabric samples for each of the four STI test runs.

STI Run Number	Log10 Reduction	Percent Reduction
1	5.63	99.99964
2	5.54	99.999986
3	5.54	99.99933
4	5.46	99.99915
Average	5.68 (SE – 0.06)	99.999

estimated during this test, because a pre-determined suspension volume was not used to load each fabric sample. However, the numerical spore recovery for the five wool samples was very high. The initial saline spore suspension count in the solution used to inoculate the wool samples was 7.00×10^7 CFU/ml. The numerical recovery is not a direct comparison in that the initial counts are based on CFU/ml of liquid suspension and the results are based on an individual sample carrier. The untreated controls and all the treated sample results are reported as the CFU counts/carrier. A carrier is defined as either a single wool fabric sample or a single wood chip sample.

The average log10 reduction for the wood chip samples was 4.72 log10 CFU/wood chip, across all four test runs (Table 8). The spore recovery from the wood chips was high, with the saline spore suspension used to inoculate the wood chip samples at 2.66×10^8 CFU/ml and the spore recovery from five wood chips retained at the MicroChem lab was 5.7×10^7 CFU/wood chip. The log10 reductions for wool and wood chips were 5.68 and 4.72, respectively. The sporicidal efficacy for the wood chips was about 1 log10 reduction lower than for the wool

samples. The lower sporicidal efficacy for the wood chips may be due to the wood chips having a lower initial CFU count (3.22×10^7 CFU/ control sample) compared to the wool samples (2.03×10^8 CFU/ control sample). Also, the differences in log10 reduction between the wool and wood chip samples show that porous surface structure of a sample is an important factor when conducting efficacy tests for disposal systems. The wool fabric has a very porous structure, but offers no thermo insulation value to the spores embedded into fabric. The wood chips also had a semi-porous surface, but the solid chips were about 0.125 to 0.25" (3.2 to 6.4 mm) thick, which may have acted as a short term, thermo insulator for any spores embedded into the chip surface. All commercial STI systems have a shredder and HEPA filter integrated into the system. The shredder on the STI system will usually produce waste particles about 7 mm in size. These smaller particles have a much higher surface area to be exposed to steam heat. The wood chips were not shredded, before they were placed in the waste bin beneath the steam auger. The wood chips were full size in order to evaluate the ability of the STI steaming system to decontaminate wood chip waste from a tree harvest operation in fruit orchards with diseased trees.

Table 8. The average *Bacillus subtilis* log 10 reduction and percent reduction for the wood chip samples for each of the four STI test runs.

STI Run Number	Log10 Reduction	Percent Reduction
1	4.46	99.9376
2	5.16	99.9964
3	4.83	99.9803
4	4.75	99.9794
Average	4.72 (SE – 0.6)	99.9733

Regression analysis for viable CFU spore counts over the average exposure time for the wood chip samples shows a significant indirect relationship (Fig. 8), which was the expected relationship. However, the same regression analysis for viable CFU spore counts over the average exposure time for the wool fabric samples shows a significant direct relationship (Fig. 9), which is an opposite relationship from the wood chips and is counter intuitive based on standard efficacy principles. The regression differences between the sample types may be a function of heat transfer rates for wood chips, and the porosity of the wool samples. This STI study had a narrow range of time of exposure (43 to 75 min.) because time was not included in the study design to be a study factor.

Sporicidal efficacy for disposal systems based on thermo technology is primarily a function of the average temperature that is maintained over a selected time period. The objectives for this study did not include temperature or exposure time as study factors. However, increasing the time of exposure may have increased the sporicidal efficacy. This may be the most practical remedy if there are concerns that sporicidal efficacy should be increased so that the wood chips were completely sanitized. Any future tests with the STI system should include a time of exposure factor in the study design, so that the relationship between efficacy and time of exposure may be quantified more accurately.

Figure 8. Linear regression between average time of exposure and average viable CFU counts for *B. subtilis* spores for the wood chip samples for the four test runs (p value <0.0001).

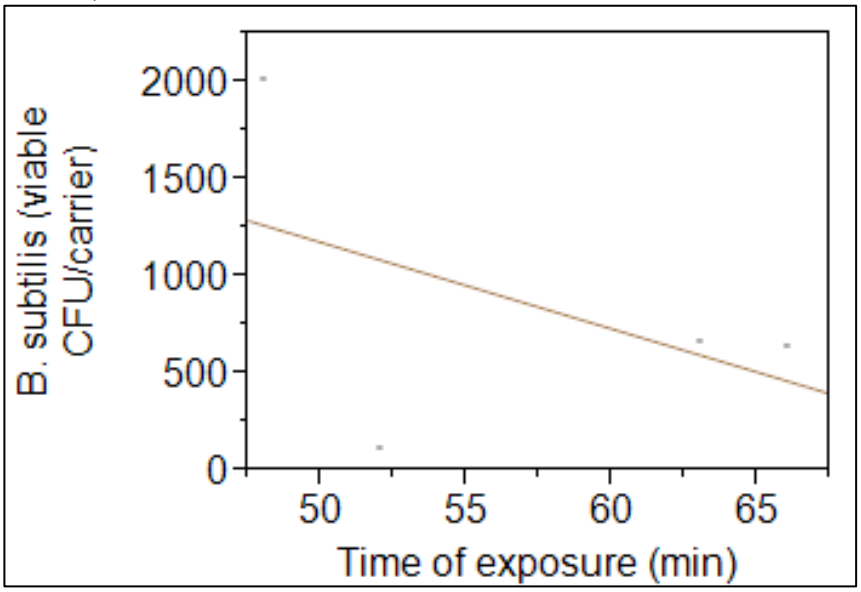
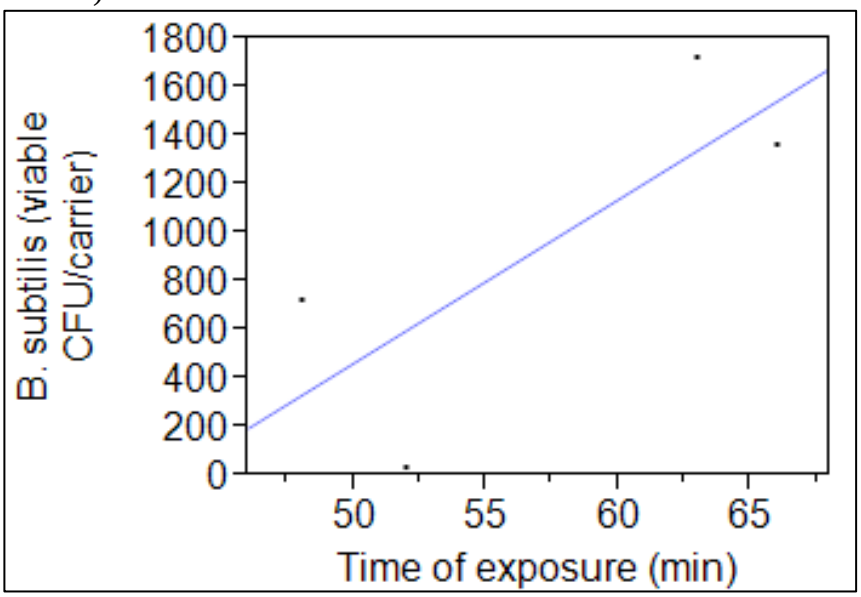


Figure 9. Linear regression between average time of exposure and average viable CFU counts for *B. subtilis* spores for the wool fabric samples for the four test runs (p value <0.0001).



Other study factors that may affect efficacy are the selection of spore forming bacteria species, how the spores are prepared and treated, study conditions, and the spore assay methods. Prepared spore samples that are not keep refrigerated can revert back into a vegetative stage, which is much easier to control with heat treatments. All of the spore samples were kept refrigerated until the day of the STI test run, and the treated samples were frozen after collection and during storage, and they were shipped with ice packs.

The sporicidal efficacy results from this STI study can be compared to the first STI study which was conducted in Oct. 2012. The results from the Oct. 2012 were summarized in an earlier final report from the CPHST Fort Collins lab. The two STI studies both included a sporicidal efficacy test using *B. subtilis* spores. However, the spore samples were different between the two studies. The Oct. 2012 study used *B. subtilis* spores enclosed in poster board and leather samples, as well as uncovered, wool/nylon fabric samples. The March 2013 study used two different *B. subtilis* spore samples: 1) spores embedded on uncovered, wool/nylon fabric and 2) spores embedded onto wood chips surfaces. Therefore a statistical comparison of the sporicidal efficacy for the two studies would be questionable due to the differences in the sample types.

The CPHST Fort Collins lab also conducted a waste disposal test with a small electric incinerator in 2013. The incinerator test also included a *B. subtilis* sporicidal efficacy test which could be indirectly compared to the results from this STI study. The average time of exposure for the wood chips was 57 min. when auger temperatures were at or above 212 F (100 C). In contrast, the electric incinerator burn times ranged from 90 to 210 minutes, with an average temperature of about 1,200 F. The incinerator study showed that it took about 150 minutes to completely burn 1,200 gm of sweet potatoes into ash. The electric incinerator burn time was about 2.6 times longer than the 57 minutes of steam heat exposure for this STI study, while only sanitizing 1.2 kg of waste. The STI system is a continuous feed design with a range of waste capacities. This STI model had a waste handling capacity of 600 lb per hour, without the slide valve gate modifications.

The USDA waste disposal standards for thermo, based technology requires the systems to reach a minimum temperature of 212 F (100 C) for 30 minutes. The STI wood chip temperature exceeded 212 F (100 C) for an average of 57 min. when averaged across all four runs. The STI shredder/steaming system met the USDA waste disposal regulation requirements for heat treatment of waste. The STI sporicidal efficacy results for the wool fabric samples show an > 6 and > 5 log10 reduction for the Oct. 2012 and the Mar. 2013 field studies, respectively. The Oct. 2012 and the Mar. 2013 STI studies were both conducted with the slide valve gates, but the wool-nylon samples were prepared and treated under different conditions. The differences in wool sample treatments should be considered when comparing the efficacy results between the two STI studies. The efficacy of any waste bio-contaminant can be increased by increasing either the temperature or exposure time. The primary method for increasing efficacy for the STI system is to control the auger speed and thereby control the exposure time of the waste treatment.

The USDA waste disposal standards do not contain any references to a generalized microbial efficacy standard or guideline. The lack of a general efficacy standard in the USDA waste disposal guidelines may allow highly resistant microbes or pathogens to not be completely destroyed, given the current standard treatment of 212 F (100 C) for 30 minutes. At least one research article has shown that prions can survive up to 1,000 C for at least 15 minutes (Brown et at. 2004). A study by Fricker et al. (2011) found that several Bacillus species could survive at 90 C for up to 120 minutes (Appendix B). A study by Fernandez et al. (1994) found that *B. stearothermophilus* spores could survive for 23 minutes, based on its D value, in a water medium heated to 115 C. The D-value is defined as 90% inactivation of treated spores, or that 10% of the spores survived at the temperature and time of exposure. These results imply that any microbial pathogens that form spores or

even pathogens that are temporarily insulated from a heat treatment may be able to survive, depending on the actual exposure time to a temperature threshold. The small size of microbes increases the probability that they may be embedded in plant or animal tissue that may form an insulation barrier, thus reducing the exposure time to a heat treatment. The ability of a disposal system to shred the waste into small particles reduces the probability of pathogens remaining embedded inside a particle. Also, smaller waste fragments reduce the inherent insulation and longer heat transfer times typical of larger particles, thereby increasing sterilization rates for waste with embedded bio-contaminates.

The primary goal of this study was to evaluate the STI system to see if it could effectively sterilize regulated garbage for potential use at USA ports, or decontaminate wood chips or soil/plant material during a domestic plant pest emergency. The STI system can be either stationary or mobile and is suitable to decontaminate a wide range of animal/plant waste, regulated garbage, soil, confiscated materials, and other contaminated materials during a domestic emergency involving plant pathogens or plant insects. The STI system can be used with or without the shredder in order to sterilize bio-contaminated soil. A secondary auger can be attached to the STI hopper to deliver soil that is loaded/scraped into a loading chute. The STI system has been used for hospital and medical clinic bio-waste disposal since 1995 without any non-compliance violations resulting in contaminated waste. The shredder/steaming waste disposal technology, such as the STI Series 2000 system, has been evaluated in three separate sporicidal efficacy studies. The efficacy results show that the shredder and steaming technology can effectively sterilize bio-contaminated waste, and therefore should be considered an acceptable heat treatment technology for regulated garbage waste disposal at USA ports and border stations.

References

Brown, P., E.H. Lemieux, B.K. Johnson, A.E. Bacote, and D.C. Gajdusek. 2004. Infectivity studies of both ash and air emissions from simulated incineration of scrapie contaminated

Fantozzi, J. A. 1995. Certifying dry kilns for European markets. Western Dry Kiln Association.

Fernandez, P., M.J. Qio, T. Sanchez, and A. Martinez. 1994. Thermal resistance of Bacillus stearothermophilus spores heated in acidifed mushroom extract. J. Food Prot. 57:37-41.

Fricker, M., J. Ågren, B. Segerman, R. Knutsson, and M. Ehling-Schulz. 2011. Evaluation of Bacillus strains as model systems for the work on Bacillus anthracis spores. Int. J. Food Microbiology. (145) pg129-136.

McDonnell, G. and P. Burke. 2011. Disinfection: is it time to reconsider Spauling? J. Hospital Infect. 78:163-170.

Meyers, S. W., I. Fraser and V.C. Mastro. 2009. Evaluation of heat treatment schedules for emerald ash borer (Coleoptera: Buprestidae) Journal of Economic Entomology 102 (6)(Proof)

Nzokou, P., S. Tourtellot and D.P. Kamdem. 2008. Kiln and microwave heat treatment of logs infested by the emerald ash borer (*Agrilus planipennis* Fairmaire)(Coleoptera: Buprestidae). Forest Products Journal 58:68-72.
Powell, J. 1950. Factors affecting the germination of thick suspensions of Bacillus subtilis spores in L-Alanine solution. J. Gen. Microbio. 4:330-338.

Rice, E.W, N. J. Adcock, M. Sivaganesan and L. J. Rose. 2005. Inactivation of Spores of *Bacillus anthracis* Sterne, *Bacillus cereus*, and *Bacillus thuringiensis* subsp. *israelensis* by chlorination. Appl. Environ. Microbiol. 71(9):5587.

Warth, AD. 1978. Relationship between the heat resistance of spores and the optimum and maximum growth temperatures of Bacillus species. J. Bacteriol. 134(3):669.

Appendix A.

The capital and operating costs and estimated carbon foot print for three STI models made by Biosafe Engineering are listed in the first table. The capital and operating costs for comparable medical waste disposal systems are listed in the second table.

Table 1. STI shredder and steamer capital cost, operating costs, and carbon footprint costs.

STI capacity (PPH= lb/hr)	Unit price	Operational costs ($/lb waste)	Carbon footprint (lbs CO2/lb treated waste)
STI Series 2000 - 300PPH	$660,000	$0.03	0.08
STI Series 2000 - 600 PPH	$722,411	$0.03	0.06
STI Series 2000 - 1000PPH	$819,247	$0.03	0.05

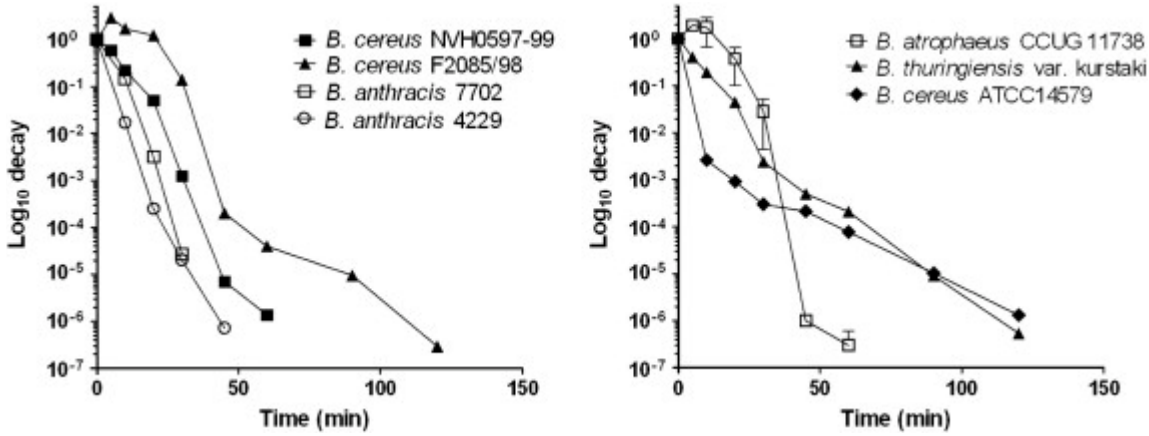
Table 2. Comparison of capital and operating costs for medical waste disposal systems, based on 2009 costs.

Disposal systems	Capital costs ($)	Operating costs ($/lb waste)
Vacuum autoclaves	75,000 - 200,000	0.11
Rotary autoclaves	400,000 - 800,000	0.12
Chemical –ozone	400,000 -1,200,000	0.10
Mobile systems	n/a	n/a
Microwave	500,000 -750, 000	0.07 – 0.10
STI	650,000 -1,200,000	0.03 – 0.07

Appendix B.

Fricker et al. (2011) conducted a spore inactivation study using temperature and time. The two graphs from his study show the inactivation (y-axis is log10 reduction in CFU counts) of five Bacillus species, in spore stage, when heat treated at 90 C for a time range of 0 to 150 min. The graphs show a difference in sporicidal efficacy/inactivation among Bacillus species over time. A 6 log reduction in initial spore counts can range for 50 min to 125 min, depending on Bacillus species. The effect of time of exposure is an important factor in testing heat treatments on sporicidal efficacy of Bacillus spores.
Disposal system efficacy results should be compared on similar temperature ranges, or on time of exposure, or at least take account for differences in temperature and time of exposure among disposal systems.

Figure 1. Inactivation of Bacillus spp. spores over time at 90 C temperature (Fricker et al. 2011)



Appendix C.

Table 1. Comparison of incineration and shredding/steamer technologies.

Incineration	STI shredder steaming system
The high temperatures (1400-1800 degrees F) associated with incineration can cause the release of acid gas and harmful particulates. To remove the harmful gases and particulate given off by the breakdown of bio-hazardous waste, modern incinerators are required to add "scrubbers" that cool the gases and collect the by- products of incineration in "bag houses" or in a wall of water in the case of a "wet scrubber." These by-products contain high levels of heavy metals and various other carcinogenic materials that must be disposed of as bio-hazardous waste. Scrubbers also require the use of expensive CEMS (continuous emissions monitoring systems) to monitor the proper operation of the incinerator and scrubber.	The STI operates in the temperature range of boiling water, achieving the same pathogen-killing results as incineration, but does not release particulate or acid gases into the air.
The incinerator typically operates for costs ranging between $0.145 and $0.20 per pound if operated in the most efficient setting of a 24-hour per day operation at full capacity.	The STI system, when operated in the same manner, has operating costs of $0.07 to $0.03 per pound when operated at peak efficiencies. Capital costs for an incinerator with scrubber and CEMS are 4-6 times higher in price than the STI Series 2000 capital costs with the same rated capacity throughput for waste.
Incinerators require highly trained individuals to operate. Their control systems are elaborate and difficult to understand. In almost every case, an engineer must be specially trained to operate an incinerator and be trained in reading opacity of the output of the incinerator.	The STI system uses simple controls that any person can be trained to use without difficulty due to its one button operation.
If users need to work on an incinerator, they must wait 1-2 days for the unit to cool down enough for the repairs to be made.	The STI system can be immediately turned off with a switch, and after a short cool down period the maintenance can be performed.

Incinerators melt glass which turns into slag and covers the “under-fire air” that helps the waste to burn. These air passages must be re-opened by using jackhammers, chisels and people. This work is quite dangerous and often requires replacement of the refractory lining of the incinerator. When the glass is chipped away, the refractory lining breaks off and must be replaced because to protect the steel skin on the incinerator. Additionally, molten glass can seize the ram feeder and the ash hoe in the incinerator. Incinerators must be operating constantly, burning expensive fuels like natural gas and oil.	The STI system can be turned on or off as needed with no need to buy fuel.