

## Investigation of the Benefits of Using Direct Steam Injection in Effluent Treatment Systems

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### Abstract

*In high-containment facilities the treatment of biological waste is very important. Liquid waste from containment level 4 laboratories and containment level 3 laboratories which handle nonindigenous animal pathogens is collected and treated in effluent treatment vessels. These vessels decontaminate the effluent using indirect steam via a steam jacket to heat the liquid to a minimum of 121°C. In some containment facilities the effluent is decontaminated by using direct steam injection to heat the load to the decontamination set point. This method of injecting steam directly into the load has the potential of providing agitation and reducing the amount of time necessary to heat the load to the set point, thereby reducing the processing time and increasing system capacity.*

*To investigate the benefits of direct steam injection, one of the effluent treatment vessels was modified so that direct steam could be used to supplement the indirect heating of the effluent. After functional testing was conducted to ensure the proper operation of the steam injection, tests were conducted to determine the efficacy of decontamination. For these tests the liquid load was spiked with bacterial spores and samples were taken during the warm-up process as well as during the decontamination period to determine when inactivation of the spores was achieved. To date, very little data have been published on the efficacy of effluent treatment vessels or comparing different methods of heating in these vessels.*

### Introduction

In recent years research and diagnostics of emerging infectious diseases and agents of bioterrorism has increased, which has resulted in a rapid expansion in the number of high-containment facilities in the world. Due to the increased risk of the organisms handled in high-containment laboratories, increased safety protocols are required before waste from these facilities can be released into the public waste stream.

All waste coming out of containment laboratories must be decontaminated; this includes biohazardous

waste, garbage, and liquid (effluent) waste. Large volumes of liquid waste may be generated due to the chemical and personal showers necessary upon exiting high containment. Liquids that drain from sinks, showers, autoclave chambers, and from washing down animal cubicles also contribute to the amount of liquid waste that must be treated before being released into the public sewer system.

Standards and guidelines in Canada require that all containment level 4 laboratories and containment level 3 laboratories which handle nonindigenous animal pathogens must have liquid effluent decontamination systems (Public Health Agency of Canada, 2004). International rules also stipulate the need for effluent treatment systems in high-containment laboratories (Mani & Langevin, 2006; Palani, 2006).

Effluent decontamination systems can utilize thermal decontamination, chemical decontamination, or a combination of the two, with thermal being the most common form. Thermal decontamination parameters vary based on the targeted microorganisms and the local regulations with which the facility must comply. Temperatures can range from 90°C-141°C with treatment times from 20 minutes to 2 hours (Mani & Langevin, 2006).

Thermal effluent treatment systems vary in design and operation. Effluent waste from containment laboratories can be collected in a receiving vessel later to be transferred to the treatment vessel or can be collected directly into a series of redundant treatment vessels. Decontamination can occur in a batch process or by continuous flow decontamination (Edwards, 2002; Mani & Langevin, 2006). During a general effluent decontamination cycle, the temperature of the effluent is raised to a set point and held at this temperature or above, for the specified period of time, after which the treated waste is cooled before releasing it into the municipal sewage system (Mani & Langevin, 2006; Palani, 2006).

Thermal effluent treatment vessels are generally heated to a set point using steam. Steam can be injected into a steam jacket located between the inner shell and the outer jacket of the vessel, utilizing the entire shell wall as a heating surface (Palani, 2006). This method is commonly known as indirect steam heating. Effluent treatment vessels can also be heated by inject-

ing steam directly into the liquid load. Direct steam injection has the advantages of more rapid heating and agitation of the liquid (Palani, 2006). The aim of this study was to investigate the benefits and efficacy of an effluent treatment vessel employing indirect heating supplemented with direct steam injection to heat effluent waste, while comparing it to the current system which employs only indirect steam heating. As part of this study, a protocol to validate the efficacy of effluent treatment systems was developed and experiments were conducted to determine the heat resistance of potential organisms for use in the validation protocol.

## Materials and Methods

### Heat Inactivation of Spores

Heat resistance was compared for three species of *Bacillus* spores: *Bacillus thuringiensis* (the spores used to test the efficacy of the effluent treatment system), *Bacillus atropheus* ATCC 51189 (an organism commonly used in biological indicators for dry heat), and *Bacillus anthracis* ATCC 4229 (representing the most resistant pathogenic organism in high-containment laboratories), as well as *Geobacillus stearothermophilus* (an organism commonly used in biological indicators for wet heat). Given that *Bacillus thuringiensis* spores are not commonly used as a biological indicator, these experiments were conducted to compare the heat resistance of the above-mentioned bacterial spores to ensure that *Bacillus thuringiensis* spores are appropriate for testing the efficacy of the effluent treatment system.

*Bacillus atropheus* ATCC 51189 and *Bacillus anthracis* ATCC 4229 spores were produced by inoculating organisms in sporulation media (10% Columbia broth with 0.1%  $MnSO_4$  solution [10mM] in saline) and incubating for 72 hours at 37°C in an orbital shaker at 150 rpm. The spore suspension was washed three times by centrifugation and then resuspended in 1/10 the original culture volume of deionized distilled water. The spore suspension was then heat shocked at 80°C for 10 minutes to kill any vegetative bacteria (Springthorpe & Sattar, 2005). The concentration of spores was determined by filter plating serial dilutions and adjusted as necessary. Dipel 2x biological pesticide (Valent Biosciences Corporation, Walnut Creek, CA) was used for *Bacillus thuringiensis* spores. The concentration was determined by resuspending the spores in sterile water and filter plating serial dilutions. Spores of *Geobacillus stearothermophilus* were obtained from Prospore ampoule biological indicators (Raven Laboratories, Omaha, NE). Prospore ampoules were opened and the contents transferred to a centrifuge tube; spores were pelleted by centrifugation and resuspended in deionized distilled water. The concentration of spores was determined by filter plating serial dilutions and adjusted as necessary.

Spore heat resistance was first tested using a Hira-yama stand-alone autoclave, model HV110 (Kasukabe-

shi, Saitama, Japan). The minimum temperature and time exposure on this autoclave is 105°C for 1 minute. Spore heat resistance of the three *Bacillus* species was tested using spore suspensions and dried spores. For suspension tests, 1 ml containing  $10^6$  spores in a micro-centrifuge tube was tested in the autoclave. For dried spore tests, spores (10 ul containing  $10^6$  spores) were dried on stainless steel carriers, transferred to Teflon vials, and placed in the autoclave. The autoclave cycle was run at 105°C with an exposure time of 1 minute. The cycle was immediately aborted after the 1-minute exposure and samples were removed. Dried spore samples were eluted in saline by vortexing. Samples were serially diluted in saline, plated on trypticase soy agar using vacuum filtration, and incubated at 37°C for bacterial growth. Growth was observed for 7 days, with initial colony counts taken after 24 hours of incubation. Spores of *Geobacillus stearothermophilus* are known to be very resistant to wet heat, so experiments were initially conducted at 121°C. A spore suspension in deionized distilled water at a total concentration of  $10^6$  spores was autoclaved in a glass test tube at 121°C for 2, 4, and 8 minutes. Each time point was conducted separately and the autoclave cycle was immediately aborted after exposure. Samples were immediately removed, serially diluted in saline, plated on trypticase soy agar using vacuum filtration, and incubated at 56°C for bacterial growth. Growth was observed for 7 days, with initial colony counts taken after 24 hours of incubation.

Spore heat resistance of the three *Bacillus* species was also tested using a PCR thermocycler, Mastercycler EP Gradient S (Eppendorf, Westbury, NY). The thermocycler heats tubes from the bottom as well as the top ensuring even heating. The thermocycler heating block and lid were allowed to reach a temperature of 99°C and then PCR tubes containing 100 ul of spore suspension, at a concentration of  $10^6$  spores/100 ul, were placed in the heating block and the lid was closed. After exposure time points, the samples were removed from the thermocycler. Samples were serially diluted in saline, plated using vacuum filtration, and incubated at 37°C for bacterial growth. After incubation of 24 hours, colonies were counted to determine the reduction in bacterial spores. Growth was observed for 7 days to monitor any changes in colony counts. All tests were carried out in triplicate. Colony counts were graphed and used to calculate the decimal reduction value (D-value). The D-value is the amount of time needed to inactivate 90% or  $1 \log_{10}$  of the microbial population.

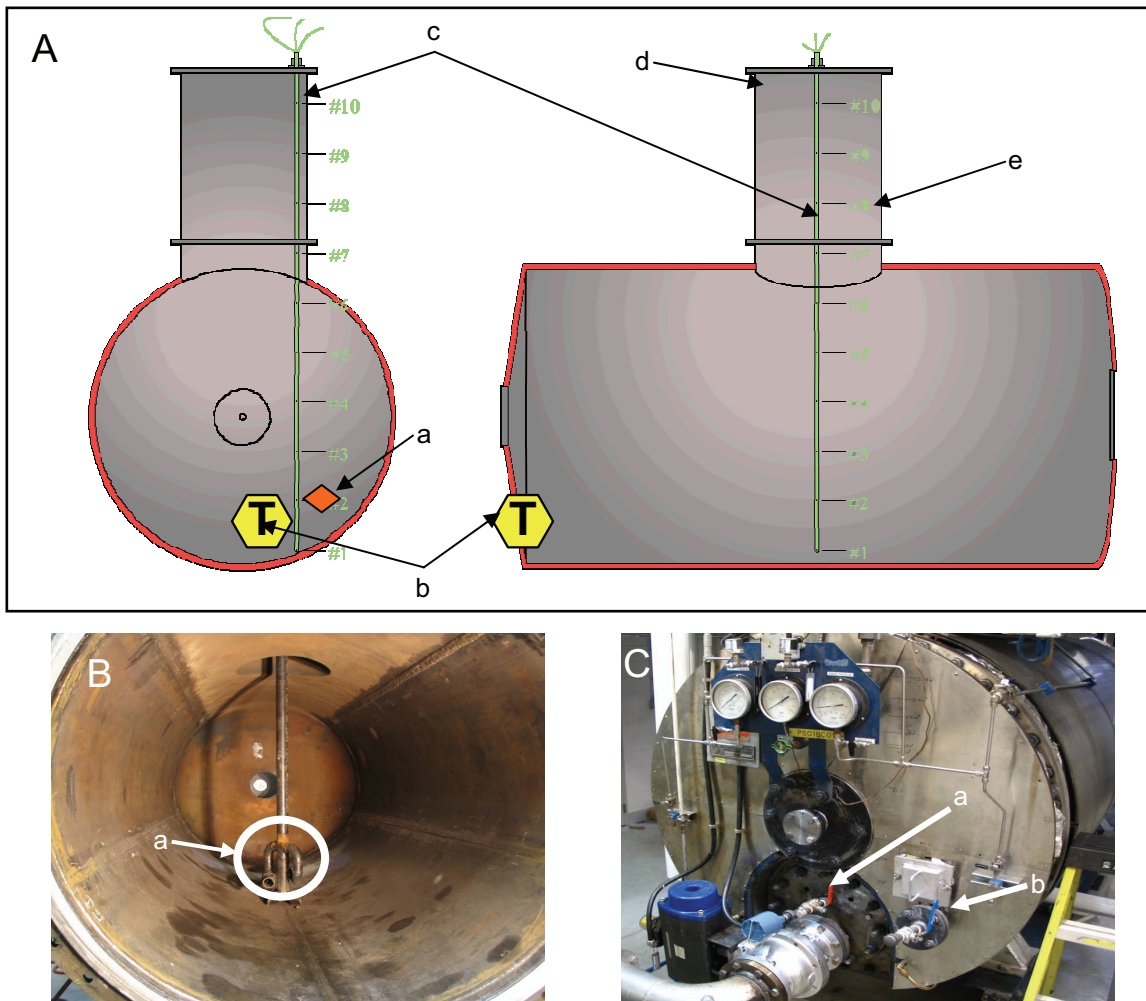
### Effluent Treatment System

The effluent treatment vessels are jacketed horizontal cylindrical vessels with a neck fitted midway to accommodate filling, venting, and other requirements. The steam injection piping was inserted from the neck and consists of two offset eductors pointing in opposite directions towards the ends of the vessel (Figure 1). The

## Figure 1

Schematic and pictures of the effluent treatment vessel.

- A. Front and side view schematic of the effluent treatment vessel: (a) sampling port; (b) dual element temperature probe for controlling the process; (c) 10-point temperature probe for heat stratification tests; (d) entry point for spore solution; (e) entry point for water
- B. Inside view of the effluent vessel after modifications for steam injection: (a) steam educators
- C. Front view of the effluent vessel: (a) location of dual element temperature probes; (b) location of sampling port



work also involved the addition of piping, control valves, and revised control logic.

Testing was conducted to ensure the proper operation of the direct steam injection; this consisted of ensuring that the vessel shell was adequately warmed up prior to initiating direct steam injection and that the rate at which the steam injection valve opened would not create severe turbulence within the vessel. A vibration switch was mounted on the vessel base and was programmed to turn off the steam injection if excess vibration was detected. A 10-point temperature probe was inserted from the neck to test for temperature stratification (Figure 1). This probe was used to monitor the temperature at 10 different locations during the effluent treatment cycle to ensure all areas of the vessel were above the decontamination set point. Also seen in Figure 1 is

the location of the dual element probe which takes an average temperature and is used to control the process. A sampling port on the vessel is in close proximity to the dual element probe to allow sampling throughout the treatment cycle (Figure 1).

### Effluent Treatment Efficacy Testing

Efficacy testing was conducted using Dipel 2x, a biological pesticide composed of spores of *Bacillus thuringiensis* subsp. *kurstaki* strain HD-1. The dry spore powder was dissolved in sterile water and the concentration was determined to be  $5 \times 10^{10}$  spores/gram. Efficacy testing was conducted using the current method of indirect steam heating and the new method using indirect steam heat supplemented with direct steam injection. For each method, tests were conducted three times on

separate days. In general, effluent treatment vessels are heated to a decontamination set point of 122°C. The temperature is monitored by the dual element probe, once the set point of 122°C is reached; a minimum temperature of 121°C is maintained for 30 minutes.

The vessel was filled with approximately 3500 ±10 litres of potable water; spore powder was added to the vessel during filling at 1500 L and 3000 L. After filling, a quick shot of steam was given to agitate the load and ensure a homogenous mixture. This yields a calculated final concentration of 10<sup>6</sup> spores/ml of effluent. At this time, a sample was taken as a positive control to determine the starting concentration of spores. The liquid load was then heated to the decontamination set point of 122°C, with samples taken every 12 minutes during the warm-up phase. Once a temperature of 122°C was reached, a minimum temperature of 121°C was maintained for 30 minutes. Samples were taken every 2 minutes during the 30-minute decontamination phase. Prior to taking each sample, the sampling port was purged to ensure samples were taken directly from the vessel.

After completion of the decontamination phase, samples were taken to the laboratory for further processing. Samples were inoculated onto trypticase soy agar (TSA) plates in a biological safety cabinet using aseptic technique. Samples were incubated at 37°C and observed daily for bacterial growth for 7 days. The concentration of surviving spores was determined for samples that were positive for growth. For each positive sam-

ple, 1 ml of sample was serially diluted 10-fold in sterile saline. These dilutions were then plated on TSA using vacuum filtration. For samples showing no growth, 10 ml of sample was plated on TSA using vacuum filtration. Colonies were counted after incubation at 37°C for 24 hours to determine the log<sub>10</sub> reduction by comparing colony counts of the control. Growth was observed for 7 days to monitor any changes in colony counts.

## Results

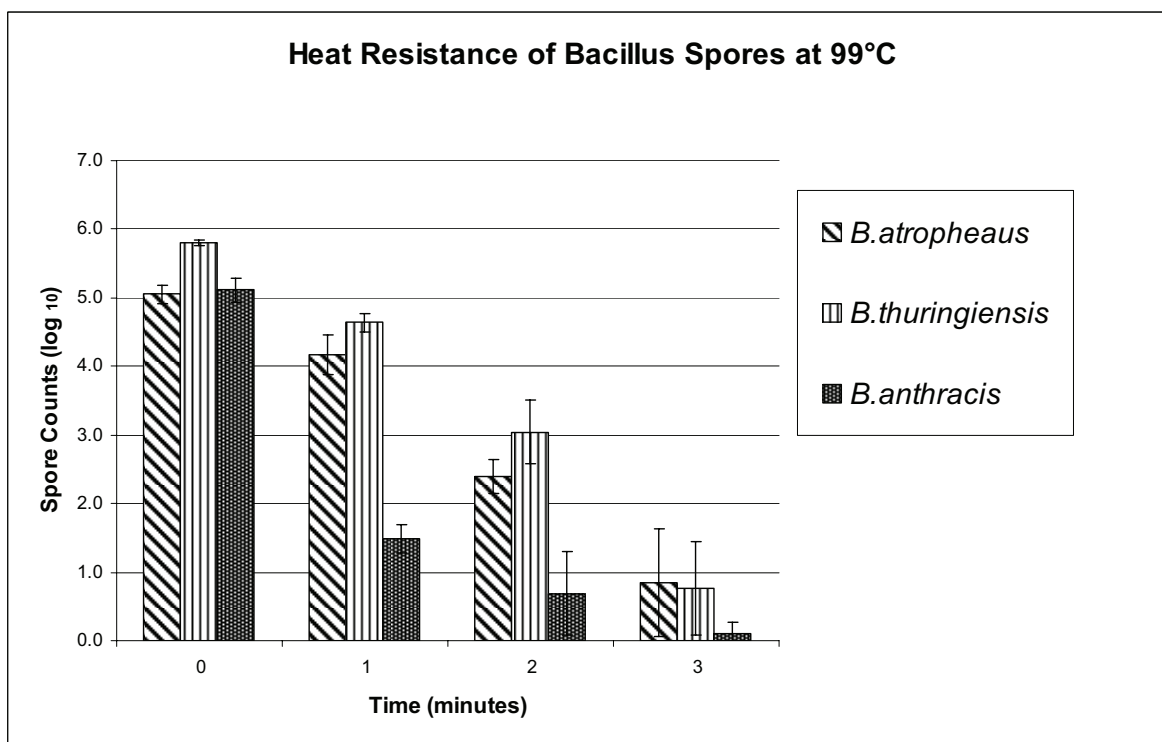
### Heat Inactivation of Spores

*Bacillus thuringiensis*, *Bacillus atropheaus*, and *Bacillus anthracis* spores were completely destroyed in the autoclave. No viable spores could be detected in either the spore suspensions or dried spores of all three species after autoclaving at 105°C for 1 minute. *B. atropheaus*, *B. anthracis*, and *B. thuringiensis* exhibited the same level of resistance to heat using an autoclave at 105°C for 1 minute. *Geobacillus stearothermophilus* was much more resistant to inactivation by autoclaving. A reduction of greater than 6 logs was observed after autoclaving at 121°C for 4 minutes. A D-value of 0.6 minutes was calculated for *G. stearothermophilus* when autoclaving at 121°C.

A steady decline in viable spores was observed for all three species tested when exposed to heat using a thermocycler at 99°C. Starting spore concentrations were between 5 and 6 logs, slightly lower than antici-

**Figure 2**

Spore counts (log<sub>10</sub>) for the three *Bacillus* spore species exposed to 99°C in a thermocycler for 1, 2, and 3 minutes compared to starting concentrations.



pated. For all three species, a 3-minute exposure to 99°C resulted in a 5-log reduction in viable spores. As seen in Figure 2, the number of viable spores declined more rapidly for *B. anthracis* than the other two species tested. Calculated D-values at 99°C for *B. anthracis*, *B. atropheaus*, and *B. thuringiensis* were 0.6, 0.7, and 0.6 minutes, respectively. The results of these experiments show that *Bacillus thuringiensis* spores have a similar resistance to heat as *Bacillus anthracis* and *Bacillus atropheaus* and are considered acceptable for use in the efficacy testing of heat inactivation.

### Effluent Treatment Efficacy Testing

For all three trials conducted using both heating methods, no viable spores could be detected in samples taken at the completion of the decontamination phase, indicating both methods were effective in inactivating *Bacillus thuringiensis* spores. Effluent treatment using only indirect steam heating resulted in an average warm-up phase of 104 ± 4.00 minutes. During the warm-up phase, a decline in the number of viable spores was observed. For two of the trials, a complete inactivation of spores was observed at 36 minutes into the warm-up phase and no viable spores could be detected after this point. For the third trial, a complete inactivation of spores was observed 60 minutes into the warm-up phase; no viable spores were detected after this point. A graphical representation of the temperature and spore concentration during the treatment cycle can be seen in Figure 3.

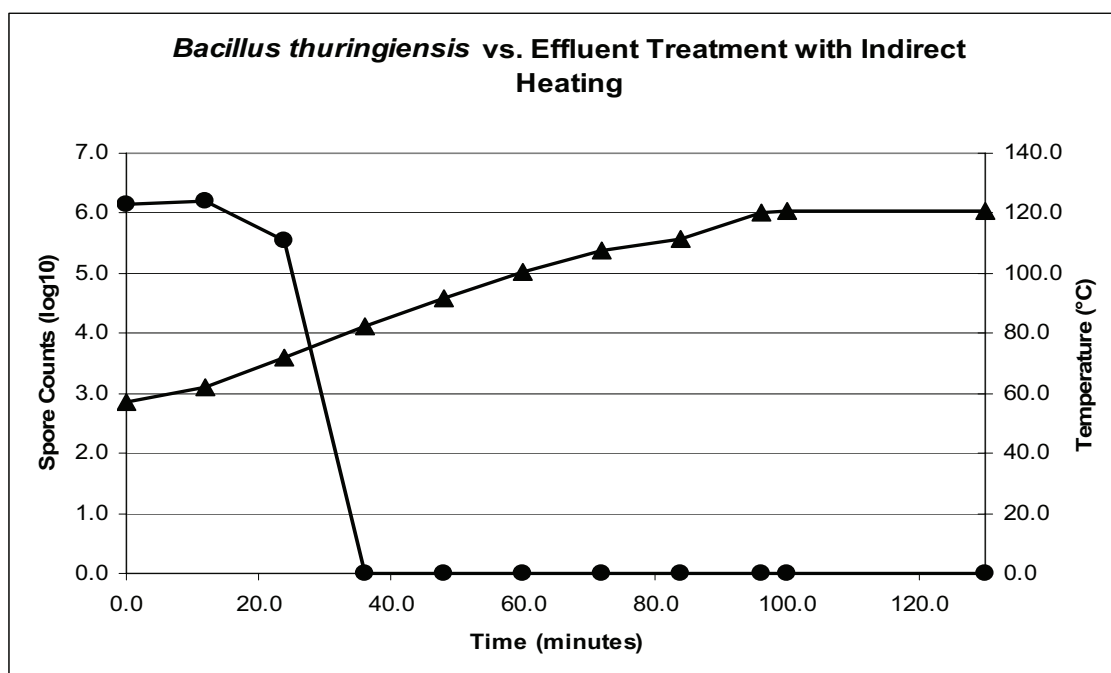
The addition of direct steam injection resulted in a much shorter average warm-up phase of 45 ± 2.52 minutes. During the warm-up phase, a steady decline in the number of viable spores was detected for all three trials. A graphical representation of the decline in viable spores during the treatment cycle can be seen in Figure 4. In the first trial, a complete inactivation of spores was observed at 44 minutes into the treatment cycle, which was 1 minute after the effluent reached the decontamination temperature of 122°C. In Trials 2 and 3, the decontamination temperature was reached a few minutes later than in Trial 1. A complete inactivation of spores was observed 36 minutes into the warm-up phase for both Trials 2 and 3 at an average temperature of 104°C.

As seen in Figure 5, both heating methods achieved a complete inactivation of spores in a similar amount of time. The major difference observed was the amount of time required to reach the decontamination set point. Using indirect heat supplemented with direct steam injection resulted in an average warm-up time of 45 minutes, while using only indirect heat required more than twice that amount of time (Figure 5).

Results from the temperature stratification tests can be seen in Figure 6. Probes 1-10 correspond to the 10 points on the temperature probe, locations of which can be seen in Figure 1. Throughout the 30-minute decontamination phase, the 10 probes recorded temperatures well above the decontamination set point of 122°C, with an average temperature of approximately 135°C. As seen in Figure 6, the dual element probe which con-

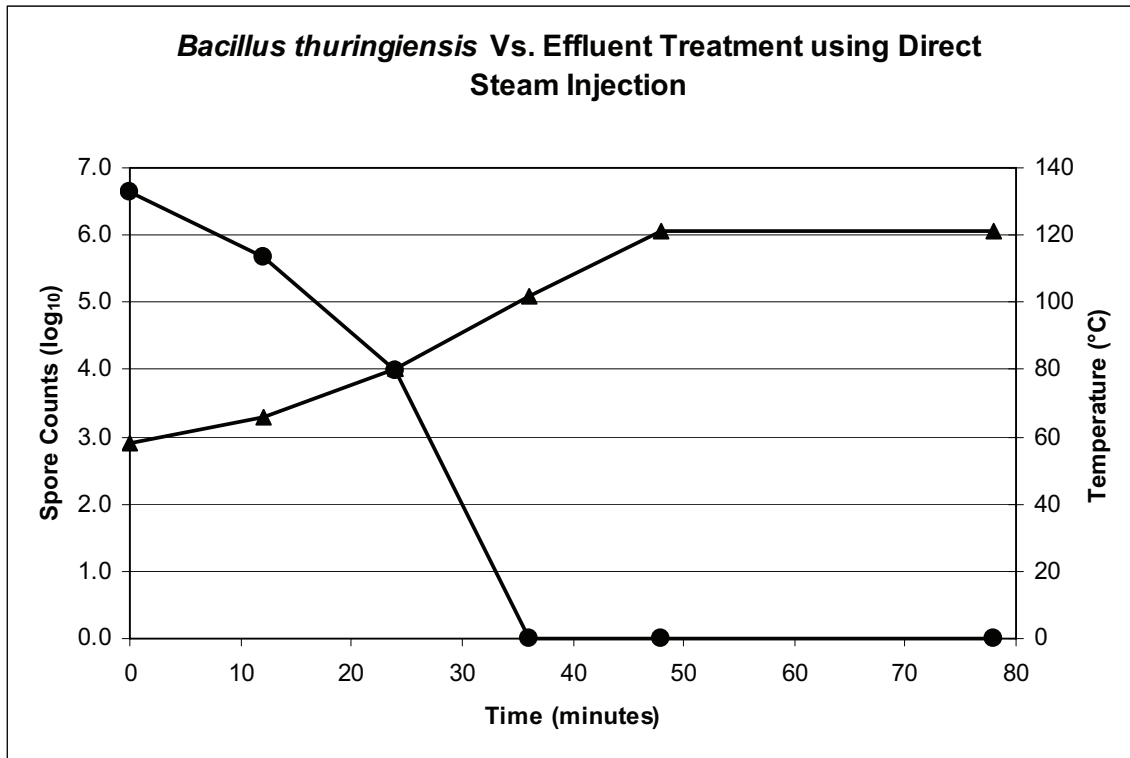
**Figure 3**

Trial 3: Temperature and *Bacillus thuringiensis* spore counts ( $\log_{10}$ ) over time during effluent treatment using indirect steam heating only. Spore counts decreased as the temperature of the effluent increased. Three trials were conducted, exhibiting similar results. (▲) Temperature (●) Spore Counts



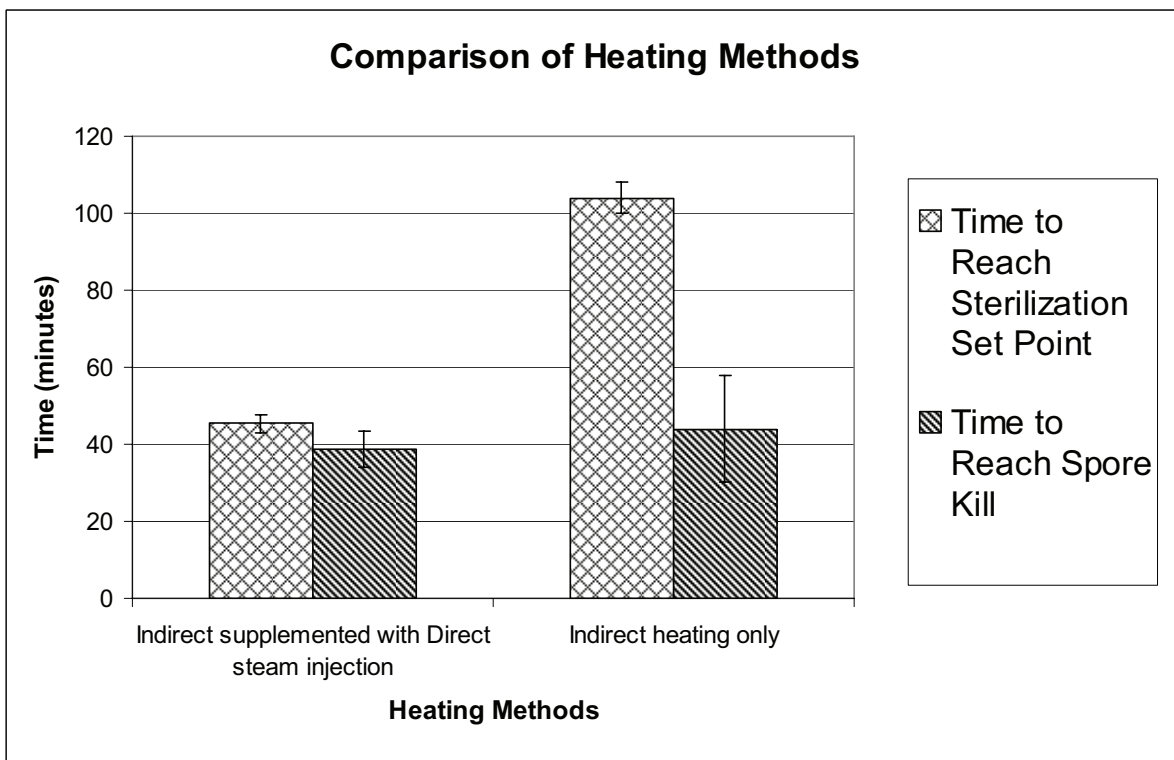
**Figure 4**

Trial 2: Temperature and *Bacillus thuringiensis* spore counts ( $\log_{10}$ ) over time during effluent treatment using direct steam injection. Spore counts decreased as the temperature of the effluent increased. Three trials were conducted, exhibiting similar results. (▲) Temperature (●) Spore Counts



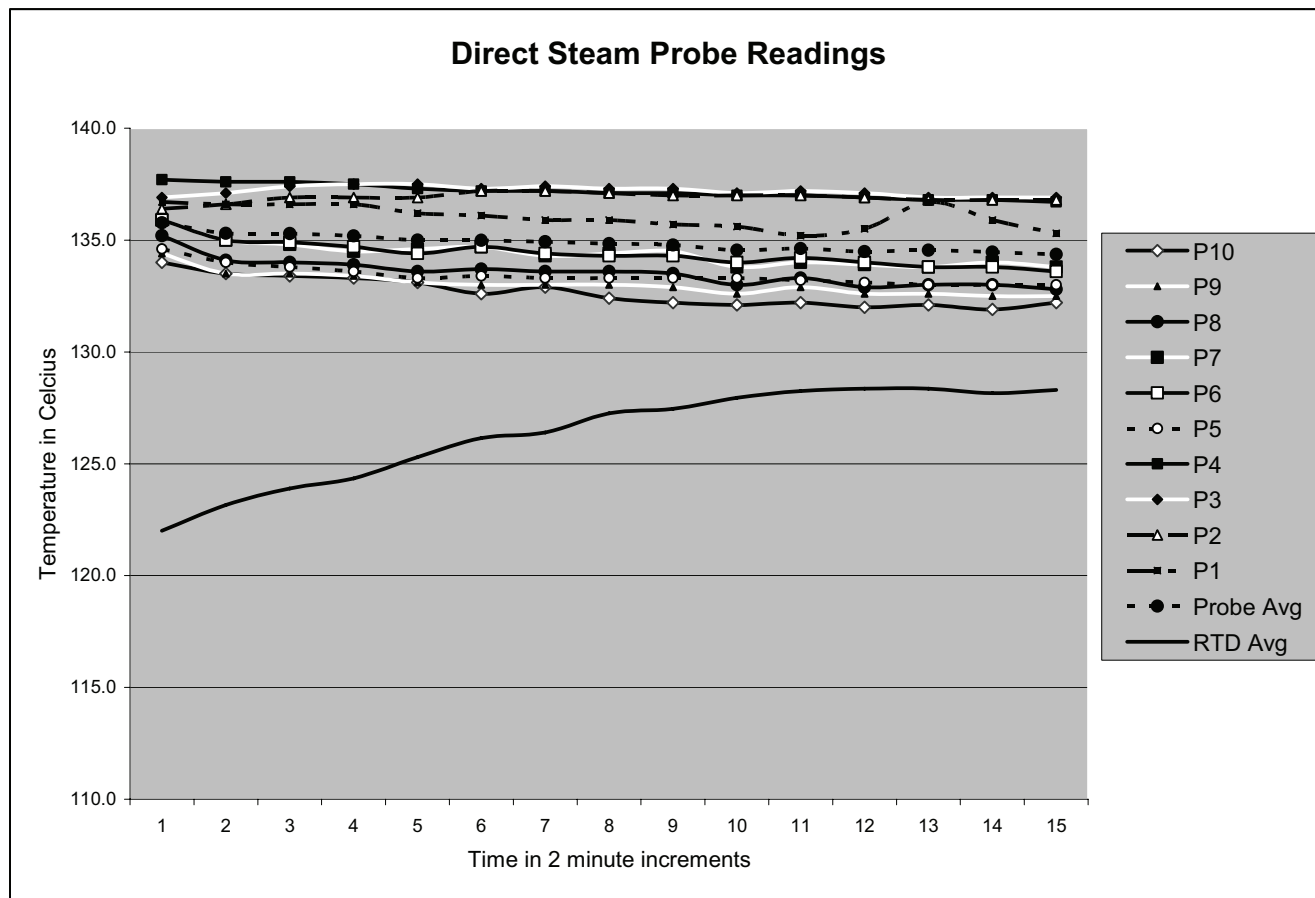
**Figure 5**

The amount of time required to reach sterilization set point ( $122^{\circ}\text{C}$ ) and to achieve a complete kill for the two effluent vessel heating methods.



**Figure 6**

Temperature stratification tests of the effluent treatment vessel using indirect heating supplemented with direct steam injection. Temperature readings during the 30 minutes decontamination phase of the 10 probes, the average of the 10 probes and the average temperature of the dual element probe used to control the process.



trols the process recorded lower temperatures during the decontamination phase. The temperature stratification results using direct steam injection were similar to that previously conducted using only indirect heating.

## Discussion

Effluent treatment processes must be validated at the commissioning of a facility or when significant changes to the process are made in order to ensure that biological decontamination of more than  $10^6$  microorganisms is achieved. This is referred to as the “sterility assurance level,” as the chances of a micro-organism surviving is one in a million (U.S. Department of Health and Human Services, 2007). The organism most commonly used for validating wet thermal decontamination is *Geobacillus stearothermophilus*, as spores of this organism are extremely heat resistant (Edwards, 2002). Spores of *Bacillus atropheus*, formerly known as *Bacillus subtilis*, are resistant to dry heat and many other decontamination methods and are often used in biological indicators to validate decontamination methods (Wittmeier, 2004).

Very little published data exist on efficacy testing and the validation of effluent treatment systems. One published study used glass vials of *Geobacillus stearothermophilus* spores placed in a long tube inside the treatment vessel (Edwards, 2002). Direct inoculation of the effluent with the test organism was the testing method chosen for this study, as it best represents the environment infectious organisms in high-containment effluent would experience. In this method, the test organism is fully submerged in the liquid effluent, unlike the method using spore vials where the test organism is separated from the effluent by a glass vial. One problem encountered with direct inoculation of the effluent is that growing spores of *Geobacillus stearothermophilus* or *Bacillus atropheus* to the high concentrations necessary for efficacy testing of such large loads is difficult and can be quite costly. This is often the reason why spore vials are chosen as a validation method over direct inoculation (Edwards, 2002). *Bacillus thuringiensis* spores were investigated for use in effluent treatment efficacy testing, as they were easily acquired at a high concentration in the form of a dry biological pesticide. Given that *Bacillus thuringiensis* spores are not com-

monly used for the validation of decontamination, experiments were conducted to compare the heat resistance of spores of *Bacillus thuringiensis* with *Bacillus atropheaus*, *Bacillus anthracis*, and *Geobacillus stearothermophilus*.

Experiments conducted using an autoclave to compare the heat resistance of the three *Bacillus* spore species resulted in complete inactivation of the three species tested. It is important to note that although the spores were exposed to 105°C for only 1 minute, they were exposed to heat for much longer, as one must include the amount of time required to heat the autoclave to the set point. It is likely that this warm-up time greatly contributed to the inactivation of the spores. Results from these experiments lead one to believe that the effluent treatment cycle should also be successful in completely inactivating *Bacillus* spore species. The effluent treatment system has a higher set point temperature (121°C compared to 105°C) and a longer exposure time (30 minutes compared to 1 minute) in comparison to the autoclave tested. As expected, *Geobacillus stearothermophilus* was much more heat resistant and a D-value of 0.6 minutes was calculated using the autoclave at 121°C. This method of heat inactivation did not provide the necessary data to compare the heat resistance of the three *Bacillus* spore species in question, as complete inactivation of all three species was found at the lowest possible temperature and exposure time. Also, it is not possible to place the spores in the autoclave after the sterilization set point is reached to exclude any inactivation during the autoclave warm-up phase. Due to the limitations of the autoclave, another testing method at a lower temperature that does not incorporate the warm-up phase was necessary.

The method of using a thermocycler to test the heat resistance of bacterial spores was chosen, as this method allows testing at a lower temperature of 99°C and also allows exposure of the spores to heat once the temperature set point was reached. This method eliminates the potential for inactivation during the warm-up phase. Data from these experiments indicate the three *Bacillus* spore species tested have similar resistance to heat, with D-values of 0.6 to 0.7 minutes. Based on these findings, *Bacillus thuringiensis* was considered an acceptable surrogate to *Bacillus anthracis* for efficacy testing of heat inactivation.

Although *Geobacillus stearotheromphilus* spores are the most resistant to wet heat, spores of *Bacillus anthracis* represent the organism most resistant to decontamination that would be encountered in the effluent waste of a high-containment laboratory. The general order for micro-organisms' resistance to decontamination, beginning with the most resistant, is bacterial spores, mycobacteria, naked viruses, fungi, vegetative bacteria, and enveloped viruses (U.S. Department of Health and Human Services, 2007). Based on this pattern, an effluent treatment system that is effective in inactivating

*Bacillus anthracis* is also effective in inactivating other pathogens manipulated in high-containment laboratories. These include, *Mycobacterium tuberculosis*, Foot-and-Mouth disease, *Yersinia pestis*, and Ebola virus. Therefore, a bacterial spore exhibiting similar heat resistance to *Bacillus anthracis*, such as *Bacillus thuringiensis*, is considered an acceptable surrogate for the efficacy testing and validation of effluent treatment systems.

The effluent treatment efficacy studies conducted demonstrate that both indirect steam injection and a combination of direct and indirect steam injection were effective in achieving inactivation of *Bacillus thuringiensis* spores. The major benefit of adding direct steam injection, observed during this study, was a decrease in the amount of time required to reach the decontamination set point. The amount of time required to reach the set point was twice as long when using only indirect heat in comparison to direct steam injection in conjunction with indirect heating. A reduction in the warm-up phase resulted in a shorter overall cycle, allowing the effluent vessel to accept another load of waste in a shorter period of time. A shorter cycle in each vessel greatly increases the overall effluent treatment system capacity. In a busy high-containment facility, an increase in effluent treatment system capacity is very important, as it ensures the system is ready to handle liquid waste at all times resulting in the reduced likelihood of interruptions for the program staff.

Temperature stratification tests demonstrate that all areas of the vessel are reaching and maintaining a temperature above the decontamination set point for the duration of the cycle. The dual temperature probe that controls the cycle recorded the lowest temperature and samples were taken at this location.

Direct steam injection also provided agitation to the effluent load. This can help to ensure equal heating throughout the load (Palani, 2006). This is a much simpler method to provide agitation compared to internal mixers which would have added installation and maintenance costs. Another potential benefit of using direct steam injection is the ability to use the steam to clean the inside of the vessels. This potential benefit requires further investigation.

In conclusion, this study has provided a realistic method for testing the efficacy of effluent treatment systems. Spores of *Bacillus thuringiensis* in the form of a dry biological pesticide provide a convenient testing method. These spores have a similar heat resistance to *Bacillus anthracis* based on the D-values obtained at 99°C. The effluent treatment system utilizes a much higher temperature (>121°C) and a much longer exposure time (30 minutes) than the conditions used for heat-resistance testing, thus ensuring a successful inactivation of the spores. Also, the 10-point temperature probe indicated the decontamination set point was reached at all test points. The addition of direct steam injection to assist in the heating of effluent treatment

vessels does not significantly change the system protocols or introduce any complications. Direct steam injection provides several benefits to the overall process and is equal in efficacy to the currently used method.

## Acknowledgments

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# A Novel Approach for Conducting Room-scale Vaporous Hydrogen Peroxide Decontamination of Virulent *Bacillus anthracis* Spores

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## Abstract

*Studies have been conducted to determine the efficacy of various decontamination technologies against virulent B. anthracis and surrogate spores within small, bench-scale chambers. This study assessed an approach for evaluating room-scale (~2,700 ft<sup>3</sup>) decontamination efficacy of vaporous hydrogen peroxide fumigation against B. anthracis Ames and B. subtilis spores deposited onto porous and non-porous indoor surface materials. Approximately 1x10<sup>8</sup> colony-forming units (CFU) of B. anthracis and B. subtilis spores were dried onto galvanized metal and ceiling tile coupons and then exposed to vaporous hydrogen peroxide. The materials contaminated with B. anthracis spores were placed inside a Class III biosafety cabinet (BSC III) that circulated vaporous hydrogen peroxide from within the decontaminated room, into and out of the BSC III. Identical materials inoculated in the same manner and at the same density with B. subtilis were placed both inside and outside of the BSC III to compare decontamination efficacy. Three fumigations were conducted using two sets of cycle parameters. The first set of cycle*

*parameters for vaporous hydrogen peroxide exposure (10 minutes of conditioning at 12 g/min; 75 minutes of decontamination at 11 g/min) yielded log reductions in viable B. anthracis and B. subtilis spores ranging from 6.1 to 7.0 on all materials, while only 76% of the commercial biological indicators (1x10<sup>6</sup> CFU) evaluated in parallel were completely inactivated. The second set of cycle parameters (12 minutes of conditioning at 12 g/min; 104 minutes of decontamination at 8 g/min) yielded log reductions in viable B. anthracis and B. subtilis spores ranging from 6.7 to 7.4 on all materials and complete inactivation of biological indicators. These results demonstrate this method as a viable approach to assess room-scale fumigant decontamination efficacy against B. anthracis Ames spores.*

## Introduction

Since the intentional release of *Bacillus anthracis* spores in 2001 in the United States, studies have been conducted to determine the efficacy of various decontamination technologies against *B. anthracis* spores, which have included liquids, gels, and fumigants. Of