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Assessment of Disinfectants in Explosive Destruction System for Biological Agent Destruction LDRD Final Report for FY04

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ASSESSMENT OF DISINFECTANTS IN EXPLOSIVE DESTRUCTION SYSTEM FOR BIOLOGICAL AGENT DESTRUCTION LDRD Final Report for FY04

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ABSTRACT

Treatment systems that can neutralize biological agents are needed to mitigate risks from novel and legacy biohazards. Tests with *Bacillus thuringiensis* and *Bacillus stearothermophilus* spores were performed in a 190-liter, 1-1/2 lb TNT equivalent rated Explosive Destruction System (EDS) system to evaluate its capability to treat and destroy biological agents. Five tests were conducted using three different agents to kill the spores. The EDS was operated in steam autoclave, gas fumigation and liquid decontamination modes. The first three tests used EDS as an autoclave, which uses pressurized steam to kill the spores. Autoclaving was performed at 130-140 deg C for up to 2-hours. Tests with chlorine dioxide at 750 ppm concentration for 1 hour and 10% (vol) aqueous chlorine bleach solution for 1 hour were also performed. All tests resulted in complete neutralization of the bacterial spores based on no bacterial growth in post-treatment incubations. Explosively opening a glass container to expose the bacterial spores for treatment with steam was demonstrated and could easily be done for chlorine dioxide gas or liquid bleach.

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INTRODUCTION

The Explosive Destruction System (EDS) is a proven technology in the safe and effective disposal of toxic chemical materiel that is discovered in unexploded munitions[1]. Sandia developed the EDS as a transportable system that safely disposes of recovered chemical warfare materiel in an environmentally sound manner. The system operates in a two-step process in a sealed vessel that first explosively opens the casing and deactivates explosives in the weapon. Reagents are then added to neutralize the chemical agent. The EDS system has been successfully deployed for use by the US Army.

We are examining the possibility of expanding EDS operational capabilities to include items that contain biological agents (i.e., *Bacillus anthracis*). This capability together with the explosive opening feature of EDS would provide a simple, hands-off solution to the challenges of opening and destroying biological agents, contaminated, recovered containers and improvised biological devices. To this end we have conducted a series of tests using the EDS system to kill *Bacillus thuringiensis* and *Bacillus stearothermophilus* spores. B. *thuringiensis* is a non-pathogenic genetic relative of *Bacillus anthracis* (anthrax) that has been used extensively in the scientific literature and governmental testing as a surrogate. Five tests were conducted using three different agents to kill the spores. In the first three tests we operated EDS as an autoclave, using pressurized steam to kill the spores. The fourth test used a 10% bleach solution and the final test used chlorine dioxide for the killing agent.

EXPERIMENTAL METHODS

Experimental Set-up: A 190-liter volume explosion containment vessel was outfitted with band heaters for a total of 24kW and mounted onto a test stand. The heaters are controlled by a PID controller to maintain the internal vessel temperature at specified setpoint temperature. The test stand provides 360-degree rotation about a horizontal axis with a computer-controlled motor drive. The motor controller is capable of clockwise and counterclockwise rotation at specified speeds, and has the ability to stop at specified rotation orientations for processing, sampling and draining. Heat tracing was installed on the door feed-throughs. Figure 1 shows the test vessel mounted onto the stand and equipped with band heaters.

The EDS system is operated by placing the device or material to be destroyed inside the containment vessel. If explosive opening is required, shaped explosive charges are inserted, aligned and then electrically wired. The vessel is sealed by closing the door and tightening the clamp bolts. The shaped charges are then detonated. Disinfectant or neutralization reagents and water are added. Based on the process requirements, the containment vessel is continuously rotated and heated. Heat is added to maintain the internal vessel temperature for the specified neutralization time. After processing, rotation is stopped and gas and liquid samples are collected. The samples are analyzed to confirm complete destruction of agents prior to draining process effluents.

Samples for bacterial growth were prepared in one of the following ways. Raw samples were prepared by mixing 2ml of EDS sample solution with 5 ml of Tryptic Soy Broth (TSB). Centrifuged samples were prepared by centrifuging 10 ml of EDS sample solution at 5000 rpm for 20 minutes at 4 °C. Liquid was removed and pellets were resuspended in 5 ml of TSB. Centrifuged and washed samples were prepared by centrifuging 10 ml of EDS sample solution at 5000 rpm for 20 minutes at 4 °C. Liquid was removed and pellets were washed by resuspension in 5 ml of autoclaved DI water three times. The water was removed with a final centrifuge and pellets were resuspended in 5 ml TSB. Plates were prepared with swabs of the EDS vessel or EDS sample solution on LB plates. Prospore2 indicators (Raven Biologicals Laboratories, Omaha, NE) were push cap activated and incubated at 60 °C for at least 48 hours. All other samples were incubated at 30 °C for at least 7 days.

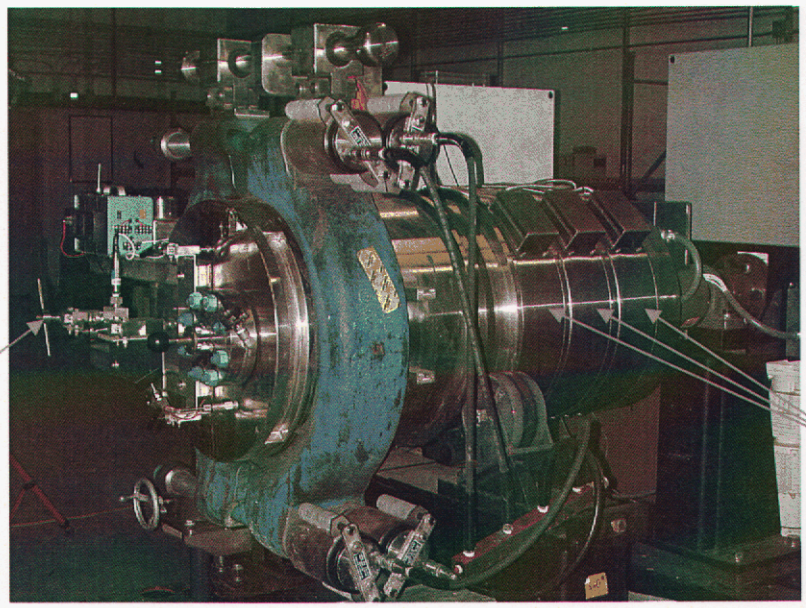

Figure 1. 190 liter EDS Test Vessel with Band Heaters

RESULTS AND DISCUSSION

Autoclave test

The primary purpose of the first test was to determine if the EDS system could reach the required 120 °C autoclave temperature[2]. The empty vessel was sealed and leak checked by

filling with 60 psig of helium. A helium leak detector was used to sniff the volumes outside the seals (door seal and high voltage feed-through seal) and showed no leakage. Sixty liters of water were pumped into the vessel. Three band heaters on the back of the vessel and heat tracing wrapped around the door feed-throughs were turned on and the vessel was rotated. The internal temperature was brought to 130 °C and was maintained there for 1 hour before the heaters were turned off and the system was allowed to cool. A 1-liter sample bottle that had been autoclaved and sealed was then attached to the vessel and used to collect a liquid sample. The vessel was drained, opened and 8 swipes were taken and cultured on LB plates at 30 °C for 5 days (Figure 2). The plates were negative for growth.

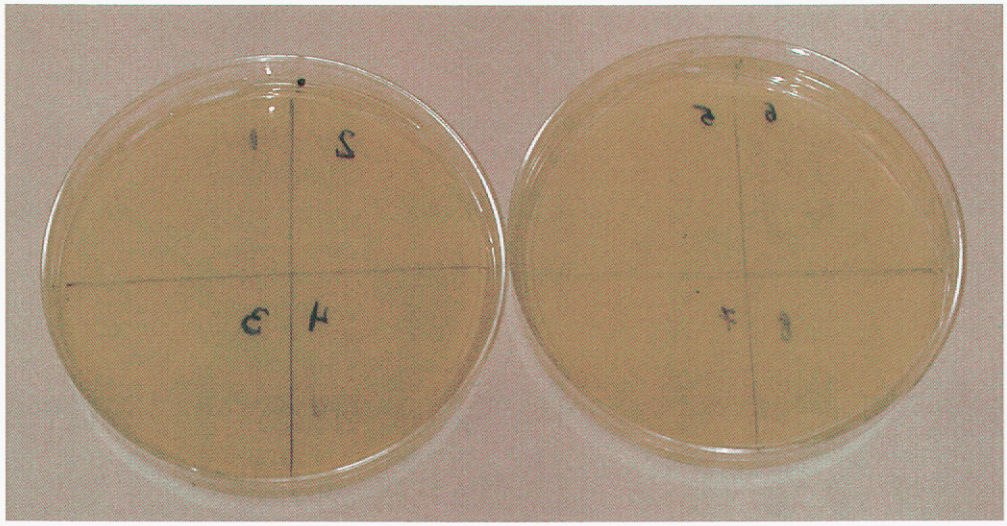

Figure 2. Luria Bertani growth media broth (LB) plates of swabs taken from first test. All plates were negative for bacterial growth after incubation for 48 hours at 37 °C.

Autoclave Test with Prospore2

For the second test we used self-contained Prospore2 biological indicator containing *Bacillus stearothermophilus* spores. This is a commercial device for testing the efficacy of an autoclave. The Prospore2 is a plastic vial with a push top cap that breaks a glass vial containing a color indicating recovery broth for a pad with spores on it. When this spore containing nutrient solution is incubated without adequate sterilization treatment at growth conditions appropriate for this organism, a color change will occur (purple → yellow) that indicates the presence of viable vegetative cells. This color change is caused by the respiration activity of the cells, which produces carbon dioxide and changes the pH of the solution into the acidic regime. If the spores are rendered inactive through sterilization, there will be no observed color change as there are no viable cells capable of producing carbon dioxide. Results are available after a 24 hour incubation at 60 °C. Seven Prospore2 indicators were placed within the EDS system, one in the door, two in the autoclave bag, and four wrapped in steel wool and loaded into a threaded pipe assembly (Figure 3) for protection. The pipes containing the Prospore2 indicators were secured in different locations within the EDS vessel in order to distribute them evenly throughout the vessel (Figure 4). The indicators were placed in multiple locations to confirm that all locations reached autoclave conditions. The vessel was sealed, leak checked with helium gas and filled with 60 liters of water. The vessel was then heated to 130 °C and rotated. The temperature was maintained for one hour before the

vessel was cooled and drained. Heating is achieved by utilizing six band heaters around the vessel for a total of 24kW. Heating tape was also wrapped around the various liquid and gas feed-throughs on the EDS door to ensure that all parts of the vessel would reach autoclave conditions. The Prospore2 indicators were removed and incubated. There was no color change in the Prospore2 indicators, confirming that the spores had been killed (Figure 5).

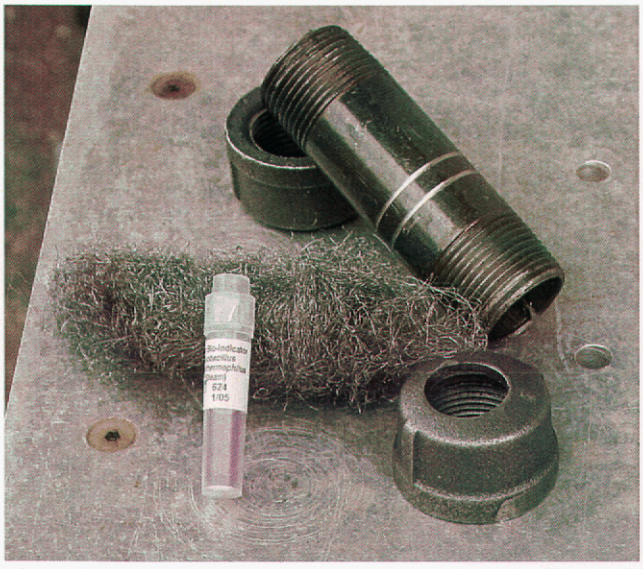

Figure 3. Prospore2 vial ready to be wrapped in steel wool and placed in pipe.

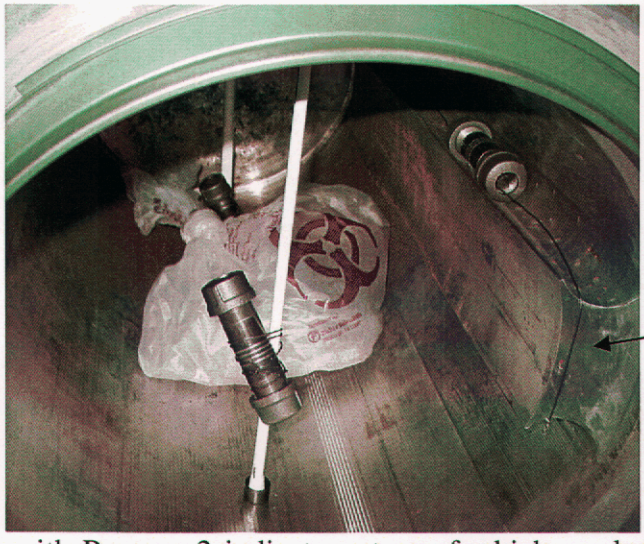

Figure 4. EDS vessel with Prospore2 indicators, two of which are located in the autoclave bag, along with two more in the door (not shown). The threaded pipes contain one indicator each.

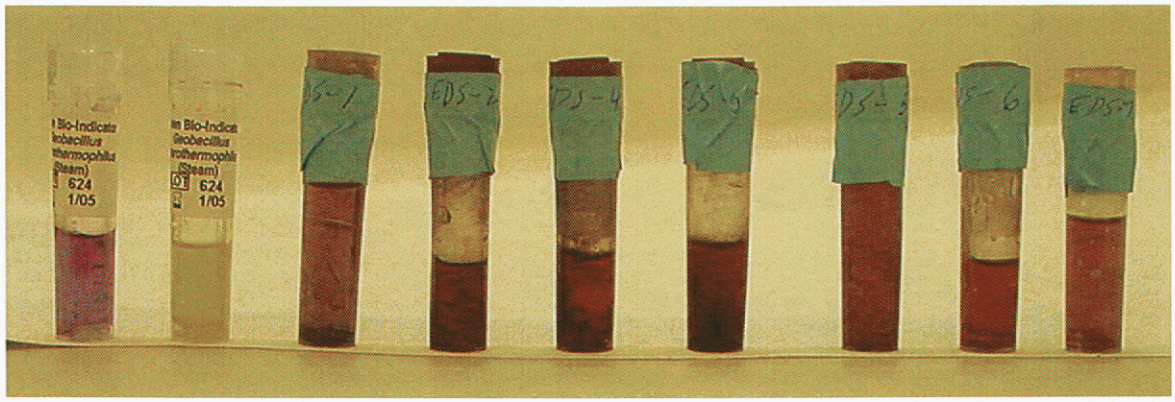

Figure 5. Prospore2 indicators from second EDS test. The two Prospore2 indicators on the left are negative and positive controls, respectively. The other seven are from the EDS test and indicate no bacterial growth.

The temperature sensor for the EDS vessel is located in the door, which is the farthest location from the band heaters and therefore the coolest location in the vessel. The discoloration of the Prospore2 indicators in Figure 5 is likely due to local hot spots in the vessel. This is also evident in the polyethylene autoclave bag that is rated to 127 °C that shrank and discolored from the heat (Figure 6).

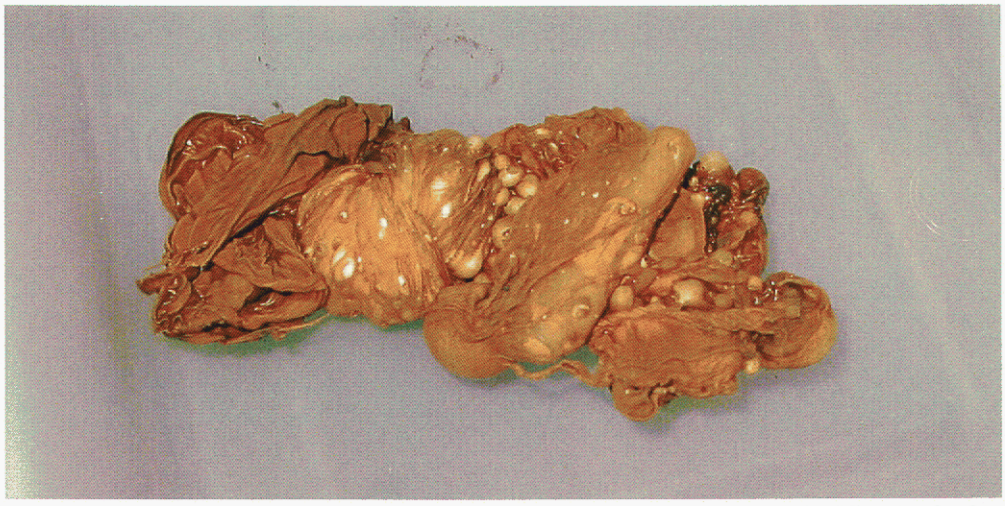

Figure 6. Autoclave bag placed in the EDS vessel (shown before test in Figure 3 above) after completion of Test 2.

Autoclave Test with B. *thuringiensis*

This experiment was conducted to test the explosive opening of a sealed container and the ability to kill spores contained within. The test used both B. *thuringiensis* spores in a sealed glass bottle and Prospore2 indicators containing B. *stearothermophilus* spores. One Prospore2 was placed in the protected location of the high voltage feed through and a second Prospore2 was wrapped in steel wool, load into a pipe for protection, and secured to the paddle. A filled 250 ml flask containing 1.6×10^7 *Bacillus thuringiensis* spores/ml was fitted with 11.2g of deta sheet and four RP-2 EBW detonators were loaded into a pipe tee for fragmentation suppression and then placed in the EDS vessel (Figure 7). The vessel was sealed and leak

checked before the explosives were detonated. Following detonation, sixty liters of water were pumped into the EDS vessel. The vessel was heated to 130 °C and held there for 1 hour under rotation. A sample bottle was filled and 8 samples (4 raw, 4 centrifuged) were cultured. Three of four that were centrifuged and incubated with growth media exhibited color change indicative of growth after 48 hours (Figure 8). The EDS vessel was kept sealed while the samples were being cultured. After receiving a positive result the vessel was heated to and maintained at 140 °C for 1 hour under rotation and resampled. The 8 samples (4 raw, 4 centrifuged and washed) were cultured at 30 °C for 5 days, and all were negative for growth. The EDS vessel was drained and cleaned. The Prospore2 indicators were recovered, incubated, and all were negative for bacterial growth.

To determine if the three positive samples were contaminated while being processed or were B. *thuringiensis* not killed in the test we plated the samples on LB for further growth and analysis. However they failed to grow and the positive controls grew as expected. This indicates that the three positive samples were not due to bacteria growth but were most like do to a pH change caused by explosives residues from the vessel. All centrifuged samples after these initial false positives include a triple wash step.

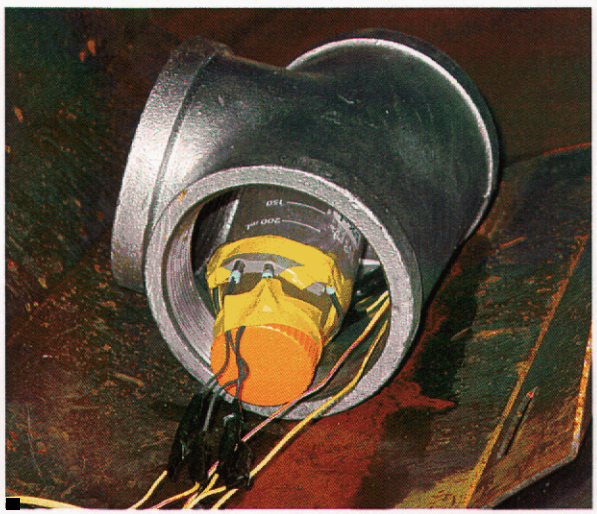

Figure 7. Flask of *B. thuringiensis* fitted with deta sheet and four RP-2 EBW detonators, loaded into pipe tee in the EDS vessel

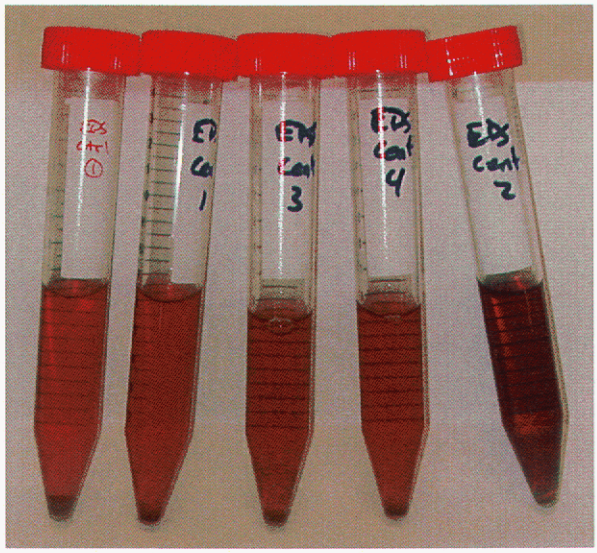

Figure 8. Picture showing the false positive results after the initial run in test 3. The left tube is a positive control. Three out of the four centrifuged samples indicate cell growth.

Bleach Test

This test used a 10% bleach solution (0.6% hypochlorite) as the kill agent. Bleach solution is commonly used as a disinfectant in academic, industrial, and government biological laboratories[3]. A filled 250 ml flask containing $1.6x10^7$ *Bacillus thuringiensis* spores/ml was placed upright in the EDS vessel with the cap loosely sitting on the bottle. No explosives were used in this test, and the bottle was setup to spill its contents when the vessel was rotated. The vessel was sealed and leak checked before 60 liters of 10% bleach in water was pumped in. No heat was applied and the vessel with its contents was rotated for 1 hour. A sample bottle was filled and 8 samples (4 raw, 4 centrifuged and washed) were cultured. All cultures were negative for bacteria growth after 6 days. After completing the culture experiments, the vessel was drained and cleaned.

Pitting was discovered in the 316 stainless steel vessel body and door immediately adjacent to the seal ring. The elongated pits were observable by unaided vision and the maximum depth was almost 1 mm. A magnified image of a silicone rubber mold of one of the pits is shown in Figure 9. The pits were only located below the level of the bleach solution (clearly delineated by staining of the vessel surface) where a crevice was formed by the contact of the seal ring and the door. No pits were observed in other wetted surfaces of the vessel. The mechanism of pit formation is attributed to crevice corrosion induced by dissolved chloride ion. The chloride ions result from reduction of NaOCl (bleach) during disinfection. Chloride ion disrupts the normally passive oxide film formed on the stainless steel in aqueous solutions. The crevice between the vessel and its seal permits locally acidic conditions to form and propagate[4]. The pH of the bleach solution was initially ~11, but decreased to approximately 6 when measured after the 6 days that the solution remained in the vessel. The decrease in pH of the solution below alkaline levels enabled crevice corrosion to proceed after an undetermined induction

period. The Grayloc seal ring, made from 17-4PH stainless steel was not available for inspection. However, it is not likely that galvanic corrosion, due to somewhat dissimilar metals in contact, was a contributing cause of corrosion.

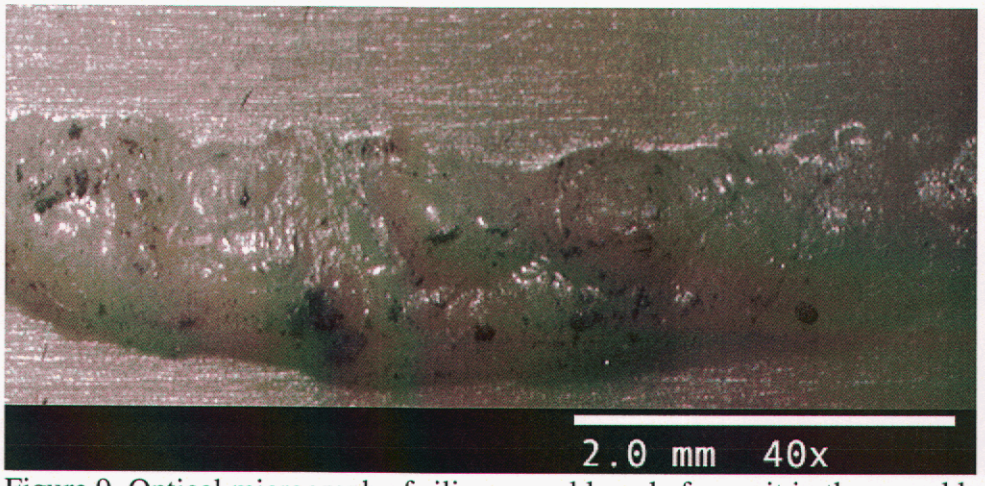

Figure 9. Optical micrograph of silicone mold made from pit in the vessel body.

Chlorine Dioxide Test

This final test used chlorine dioxide (ClO_2) gas as the kill agent[5]. The gas was generated in situ. The commercially available mixture Purate® (Pure Line Water Treatment Systems, Santa Clara, CA) was used to generate the ClO_2 by adding 90% sulfuric acid (H_2SO_4) in the following reaction. $2NaClO_3 + H_2O_2 + H_2SO_4 \rightarrow 2ClO_2 + O_2 + Na_2SO_4 + 2H_2O$. This reaction is commonly used to generate gas concentrations of 750 ppm ClO_2 or higher for decontamination applications. The sulfuric acid in a reservoir was added to the Purate® through an electrically activated valve. When the valve was opened, the sulfuric acid was gravity fed into a beaker of Purate® and generated the chlorine dioxide. The whole apparatus was placed and operated in the EDS vessel (Figure 10). An open container with 70 ml of $1.6x10^7$ *Bacillus thuringiensis* spores/ml was placed into the EDS vessel near the ClO_2 generator. The vessel was sealed and leak checked prior to generation of ClO_2. The vessel was not rotated for this test. After 1 hour, the vessel was vented and purged with helium until ClO_2 could no longer be detected with a chlorine draeger tube (Draeger Safety Inc., Pittsburgh, PA) which also detects chlorine dioxide. The *Bacillus thuringiensis* spores were plated on LB and incubated. One yellowish colored colony grew on the plate and was found to be a contaminant by Gram staining. The colony was a Gram negative cocci while *B. thuringiensis* is a Gram positive rod that formed white colonies in our controls.

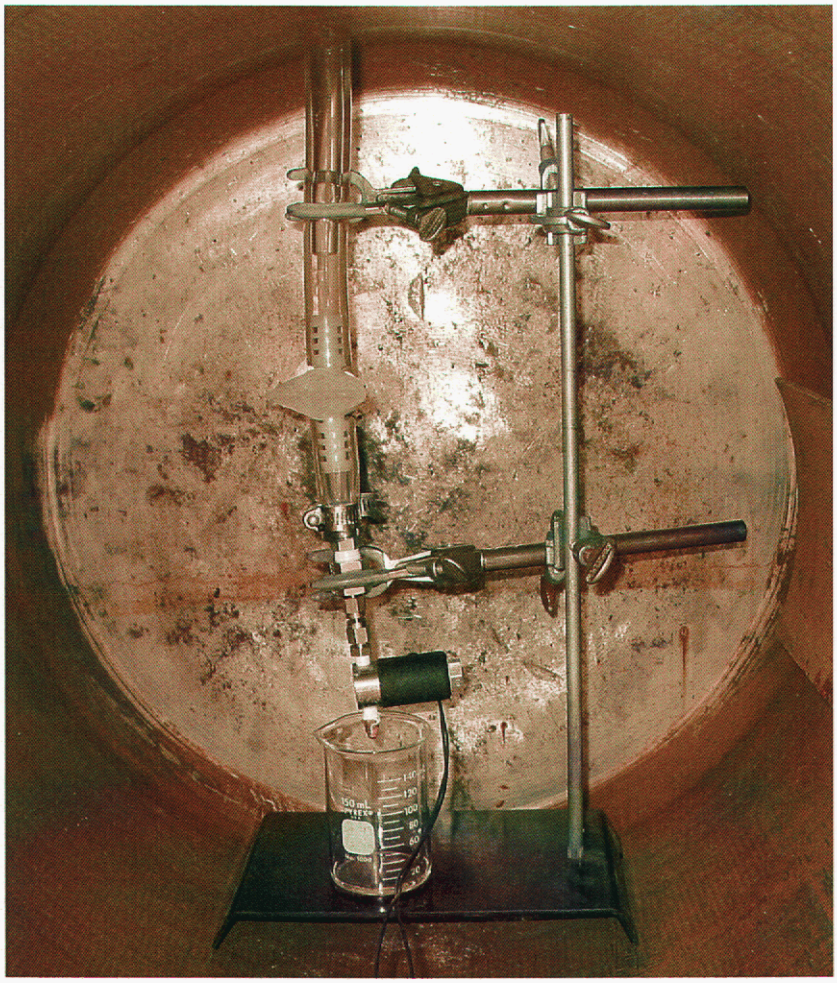

Figure 10. Apparatus for the generation of ClO2. Sulfuric acid in the upper column mixes with Purate® in the beaker when the electronic valve is actuated.

RESULTS AND CONCLUSIONS

The EDS system is effective for killing B. *thuringiensis* (a relative of anthrax) and B. *stearothermophilus* spores using any of the three treatments examined in these tests. Spores are the most resilient form of a microbe and are therefore the hardest to kill. It is therefore noteworthy that all treatments were successfully demonstrated. While all three agents worked well each has its advantages and disadvantages.

Using the EDS system as an autoclave worked well and required no additional chemicals since steam is used for the killing agent. It is important to ensure that all interior parts of the vessel reach autoclave temperatures including sampling ports and feed-throughs in the door. While this was achieved in these experiments by wrapping the feed-throughs with heating tape, we feel that a redesign of the sampling system and other feed-throughs, as well as an improved thermal design covering heating and insulation, would optimize this option.

A bleach solution was also effective as a killing agent. Added advantages to this technique are that bleach is a cheap and readily available chemical. A significant asset of the EDS system is its ability to safely contain the agent until analytical tests can confirm the agent's destruction. However the corrosive effects of the bleach limit the ability to contain the disinfectant for extended periods of time without damaging the EDS vessel. We believe this drawback can be overcome with techniques like buffering the disinfectant solution to maintain a high pH, passivating the surface of the vessel and/or changing the materials of construction. Further study beyond the scope of this project is required.

Chlorine dioxide was effective at killing the spores. Being a gas, it permeates through all the internal vessel volumes of the vessel without rotation. The high solubility of chlorine dioxide in water ensures that spores in aqueous solutions are killed, as demonstrated in this test. The use of chlorine dioxide in this test was achieved by generating the gas in situ, but for field-deployed vessels it will be more practical to use a commercial generator to make the gas and pump it into the EDS vessel. Since venting chlorine dioxide is a safety issue it will be necessary to vent through scrubbers to remove the hazard. Liquids from the vessel are likely to outgas chlorine dioxide for an extended time and will therefore need to be collected in drums protected with scrubbers. The chlorine dioxide may cause the same pitting problems seen in the test using bleach. Materials of construction for this concept will require further study.

These tests have demonstrated that the EDS successfully treats and destroys biological agents. Common killing agents and methods such as steam autoclaving, liquid bleach and chlorine dioxide are compatible with the EDS technology platform. However, each method has different process requirements and trade-offs for implementation. The next step to advance the EDS technology for use with biological agents requires further conceptual and detailed engineering and design. Depending on the process requirements, further work could include: developing designs to deliver process reagents, improving thermal control, improving sampling system hardware and selecting optimum materials of construction.

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