Impact-Driven Sporicidal Iodine Thermite Reaction

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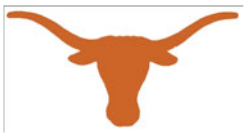

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Abstract

Biological weapons such as anthrax have caused increased national concern. Thermite reactions such as the iodine pentoxide and aluminum nanoparticular thermite reaction listed below provides a promising method for reducing thethreat of biological warfare with anthrax.

$$10\ Al + 3\ I_2O_5 \rightarrow 5\ Al_2O_3 + 6\ I$$

Thermites are a mixture of metals and metal or non-metal oxides that undergo reduction-oxidation reaction and are generally very exothermic. For the iodine pentoxide and aluminum thermite reaction, the combination of the extremely high temperatures of up to 3253K (Fischer & Grubelich 1998) from the violently exothermic nature of the reaction and the bacteriacidal iodine gas generated from the reduction-oxidation process provides great potential for neutralizaing the bacterial spores—a potential biological weapon of mass destruction. The pressure and shear stress produced from the impact-reaction serves as the driving force for overcoming the relatively high thermite reaction activation barrier.

An experimental protocol to determine the lethal effects of impact-driven thermite reactions on the survivability of *Bacillus subtilis* spores was established. Results thus far have been quite promising. Iodine pentoxide and aluminum thermite reaction conducted has been shown to negatively influence the growth

of bacterial spores, resulting in an average survival rate of 3 ± 5% after exposure to the reaction byproducts. The temperature outside of the experimental chamber was measured only to be 40 degrees Celsius. Thus, we may draw the preliminary conclusion that the thermite reaction byproducts have a greater negative influence on bacterial spore germination than temperature.

Introduction

Background and motivation

There has been an increased concern for bioterrorism threats in the recent decade. *Bacillus anthracis*, commonly known as anthrax, is one of the emerging infectious diseases that pose great potential threat as a biological weapon of mass destruction. Public awareness for the threat of anthrax was raised after six letters containing one to two grams of anthrax spores were distributed through the U.S. postal service in 2001, thus resulting in 22 cases of anthrax infections and five fatalities (Webb 2003).

Anthrax spores are on the scale of microns; once inhaled into human lungs, the spores will be transported into the blood stream and travel to the lymph nodes. Since the human body contains nutrients and meets various other requirements for growth, it provides the optimal environment for anthrax bacterial germination and replication. As the bacteria divide and spread

throughout the body, toxins are produced and may lead to severe illness and even mortality (Henderson 1999).

The high level of resistance of dormant bacterial spores to chemical and physical stress further complicates the issue of disinfection and sterilization of spores in both the fields of public health and the food industry. The dipicolinic acid in the spore structure provides bacteria with a greater ability to resist environmental stresses than that of the vegetative form. Even spores that are not killed has completely are clearly damaged may still eventually germinate (Russell 1990).

With the heightened concern for threats of anthrax bacterial spores as a biological weapon of mass destruction, the goal of this Defense Threat Reduction Agency funded research project involves the investigation of bacterial spores destruction by impact-driven advanced energetic biocidal reactive materials reactions for military applications.

The scope of the project involves the understanding of reaction kinetics of materials that generate biocidal gases, and understanding of the effect impact-generated biocidal gases and nanoparticulate metal oxide thermite reaction products on bacterial spores. I was involved in the biological experimentations of the research project. This included evaluation of lethality of impact-driven thermite reaction byproducts to bacterial spores upon exposure.

Advanced energetic thermite reactions

Reactive materials used in thermite reactions have received considerable attention in the recent years (Thadhani 1994). Thermites are composed of metals and metal or non-metal oxides that undergo exothermic reduction-oxidation reactions that generate large amounts of heat. The most famous thermite reaction is the aluminothermic reaction involving iron oxide and aluminum as listed below:

$Fe_2O_3 + 2\ Al \rightarrow 2\ Fe + Al_2O_3 + heat$

In the particular reaction, since aluminum has a higher affinity for electrons and a higher reduction potential, the transfer of electrons from the aluminum to iron oxide is extremely favorable and spontaneous. As a result, iron (II) oxide is reduced and becomes iron, while aluminum is oxidized and becomes aluminum oxide (Woodley; Hoffman 2009).

Figure 1. Thermite reaction involving iron oxide and aluminum are very exothermic in nature (Hoffman 2009).

Thermite reactions may generally be expressed in the following reaction:

$$M + AO \rightarrow MO + A + heat$$

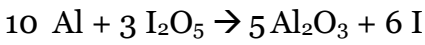
Where M is a metal, A is either a metal or non-metal, O symbolized the oxidized state (Brunskill 2009). Thermite reactions can be used in a great variety of applications, including welding of rail road tracks, fireworks, the Ames process for obtaining pure uranium, military grenades, and so forth.

Most of the investigations of impact-initiated thermite reactions have been focused on dispersion of the reacting material in air. In contrast, experimentations at the Hypervelocity Effects Division at the Institute for Advanced Technology have been focused on initiation of thermite reactions *within* the target itself.

Initiation of thermite reactions generally require heating to relatively high temperatures. Chemical characterization studies determined that the activation energy of the iodine pentoxide and aluminum thermite reaction was approximately 152 kJ/mol (Martirosyran & Luss 2009). Thus, thermite reactions will need input of sufficient input energy to overcome the activation barrier and enable the reaction to proceed. In impact-driven reactions, the intense pressure and shear stress generated by the projectile-target impact serve as the driving force of the reaction.

Bacteriacidal effect of iodopentoxide and aluminum thermites

In this project, iodine pentoxide and aluminum were selected as the thermites for the impact-driven reaction. The stoichiometrically balanced chemical reaction is shown below:



$$10\ Al + 3\ I_2O_5 \rightarrow 5\ Al_2O_3 + 6\ I$$

The particular thermite combination was chosen based on the following criteria:

1) The reaction is extremely violent and exothermic. Characterization studies indicate a value of -6.22 kJ/g for the ΔH enthalpy (Martirosyan & Luss 2009). Tremendous amounts of heat up to a theoretical temperature of 3253K with phase change have been shown in literature (Fischer & Grubelich 1998). Once ignited, the thermite reaction may propagate in a self sustaining manner (Russell, Bless, Pantoya 2010).

   Bacterial spores subjected to high temperature environments are injured by the heat exposure as indicated by significant reduction of the bacteria's ability to germinate and form colony forming units (Edwards et al. 1965). Heating anthacis spores at 100 degree celcius in moist conditions for 5 minutes or less was able to achieve complete killing; heating anthracis spores at 135 degrees Celsius in dry conditions for 10

minutes was able to achieve the same killing rate for the same anthracis strain (Murray 1931). The experimentally determined relationship between exposure time and temperature for *Bacillus thuringiensis* is shown in the figure below in Figure 2. Even at high temperatures e.g. 500 degrees celcius requires an exposure time of several seconds (Alexander et al. 1998)

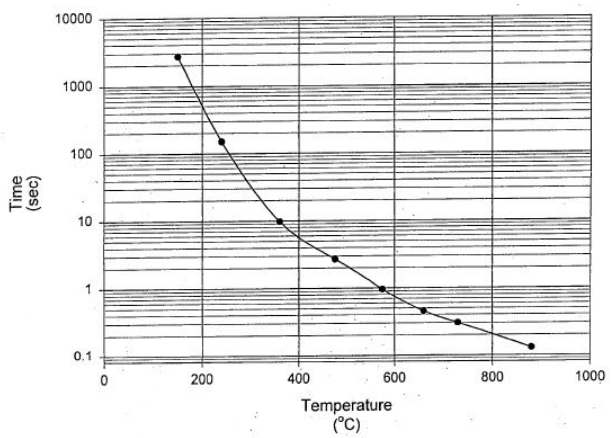


Figure 2. Exposure time vs. temperature for *Bacillus thuringiensis* (Alexander et al. 1998).

Although the spore form of bacteria is more resistant to heat than the vegetative form, it is still vulnerable to extreme high temperatures. The theoretical temperature generated by the iodine pentoxide and aluminum thermite reaction of 3253K is much higher than the experimental conditions utilized in literature although the duration period of exposure is within only millseconds. The extremely high temperature produced from the exothermic thermite reaction thus provides great potential for destroying bacterial spores.

2) The iodine pentoxide and aluminum thermite reactive materials are also appealing due to their high physical density. This makes it easier to deliver the component to a potential threat. Since iodine gas condenses as the gas expands, the spatial lethality of the thermite reaction is controllable, which is important for limited which is important to limit collateral effects extreme to . As previously mentioned, the particular reaction also has high activation energy. The relatively inertness and low chemical reactivity under standard conditions also makes the thermites safer to handle than other reaction materials that might be considered for weapon destruction.

3) One of the byproducts of the thermite reaction, iodine gas, has been shown to be bacteriacidal in a great deal of literature. Koch has even demonstrated the toxic effect of iodine to anthrax spores (Koch 1881). Iodine is used for a wide range of disinfection applications including dressing for wounds, drinking water purification, wastewater management, and air disinfection (Gottardi 2001).

    Iodine is able to penetrate the bacterial cell wall. It is suspected to cause lethality of bacteria by deoxyribonucleic acid damage, inactivation of enzymes necessary for spore germination, or alteration of the spore cortex structure which in turn disables its degradation necessary for germination (Kida et al. 2004). The damage often hypothesized to be achieved by the

following molecular biochemical mechanisms: oxidation of the thiol functional groups in biomolecules such as cystein amino acids; iodization of phenol and imidazole functional groups of tyrosine and histidine amino acids causing disrupton of protein biosynthesis and normal functioning; iodize pyrimidine derivatives cystosine and uracil of nucleic acids and prevent hydrogen bonding due to steric hindrance of the additional iodo group, potentially causing denaturation of nucleic acid; iodize fatty acids and decreasing the mobility and fluidity of lipid bilayers. These speculations are based on evidence of disrupted phospholipid layers in the cell wall under the electron microscope (Gottardi 2001).

The combination of extremely high temperatures and bacteriacidal byproducts from the impact-driven thermite reaction of iodopentoxide and aluminum thus provides great potential for destroying bacterial spore biological weapons.

Scope of thesis

The objectives of the efforts include the following:

1) Demonstrating inactivation of bacterial spores exposed to biocidal thermite products of the iodine pentoxide and aluminum reduction oxidation reaction.

2) Qualitative measurements of impact-driven nanoparticulate metal oxide thermite reaction products effects on spores and exposure parameters necessary to inactivate bacterium

The project was further divided into two components: phenomenology of the chemistry and biology after the impact-driven thermite reaction, and quantitative understanding of the lethal mechanism for the destruction of spore-forming bacteria.

My responsibility is focused on the latter biological screening tests. This included determination of the lethality and effect of impact-driven thermite reaction byproducts on the viability of spore-forming bacteria.

While the impact-driven reactions were conducted at the Institute for Advanced Technology, static combustion tests were done by our collaborator Michelle Pantoya in the Mechanical Engineering department at Texas Tech University. Combustion tests involved chemical and physical characterizations of the thermite reactions via spectroscopic measurements; determination of lethality to spore-forming bacteria from exposure of combustion prodicts; development of quantitative data for combustion kinetics of selected materials.

Experimental Parameters

Biological specimen

Instead of using the extremely hazardous and infectious *Bacillus anthracis*

for our laboratory experiments, *Bacillus subtilis* was the bacterial strain tested instead (SGM biotech, inc). The bacteria was suspended in 40% ethanol mixture to prevent aggregation of spores.

For the impact-driven reactions, the spore suspension was pipetted into tubes and allowed to dry overnight. Several types of tubes were evaluated. Scintillation tubes were selected because of their wide mouth, convenient size, and flat bottoms. Due to the hydrophobic nature of the spores, the majority of them will adhere to the bottom and sides of the tubes. These scintillation tubes were used as the containers for holding the spores within the experiment chamber.

Germination experiments

The first stage of the experimentation was the determination of germinating population from the purchased bacterial spore suspension. Serial dilutions was made and each dilution was spread over agar plates for quantification of resulting colony forming units after a 24 hour incubation period at 37 degrees Celcius, which is the optimal condition for bacterial germination and growth in the vegetative form. Theoretically, 100% of the bacterial spores should germinate. The following dilutions were plated in the germination control experiments: 10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8}.

Chemical reactant exposure experiments

To eliminate the variable of incomplete reaction and residual reactants, aluminum and iodine pentoxide must be plated together with the bacterial spores. We must establish whether or not agar contaminated with the reactants—aluminum and iodine pentoxide—will support the growth of bacteria. About 10 to 100 mg of alumina were weighed out, suspended in 100 μL of sterile water, and plated onto agar plates before the bacteria spore suspension was spread over the agar.

Similarly, iodine pentoxide powder was mixed with sterile water until the chemicals were homogenously suspended or dissolved into solution. The iodine pentoxide suspension was also spread evenly over the agar surface before plating of bacterial spores.

Impact experimental setup

After a series of experimentation with the chamber setup including utilization of self-sealing polymers in attempt to enclose the opening in the chamber caused by the projectile puncturing, it was decided that the best approach was to connect the barrel of the gun to the chamber and prevent spore escape into the environment.. This will prevent gas leakage and enable control of experimental parameters, such as exposure time.

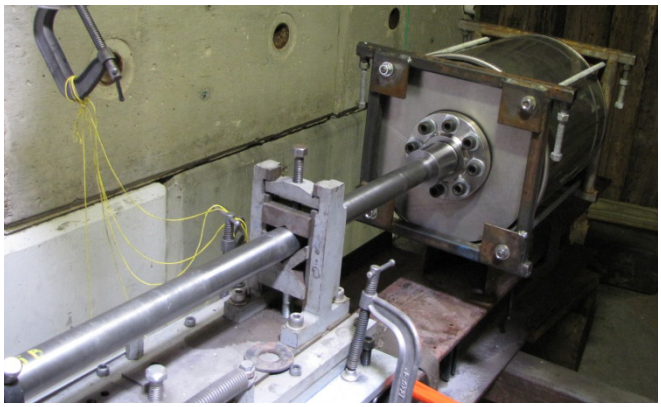


Figure 3. Experimental setup with the 0.50 caliber gun barrel connected to the experimental chamber.

The projectile used in the experiments was a 0.5 inch brass cylinder that mated to a 0.50 caliber case and smoothbore 0.50 caliber gun barrel. About 10 grams of thermite reactants were used, with approximately 7 being iodine pentoxide and 3 being aluminum. The projectile had a total weight of around 20 grams. This was shot at a velocity of 1 km/s and impacted a 35 × 35 × 4.3 mm steel strike plate. The charge in the breech was 14.4 grams of smokeless BMG 50 gun powder.

The experimental chamber was an 8 gallon steel drum. In order to maintain the structural integrity of the experimental chamber, the end of the steel drum was clamped within an exterior steel frame. Within the chamber, there was another steel frame composed of three 9.75-inch squares and a total height of 15.5 inches. The figure below shows the steel square frame. The back square had a steel back plate welded on to prevent puncture through the back of the experimental chamber. The strike plate was welded onto the middle square of

the steel frame. Note that in this picture, the frame is shown to be vertical but it was shot in the horizontal position.

Figure 4. Steel frame of squares that was placed within the steel drum experimental chamber. Both the strike plate and back plate were welded onto the steel frame.

In initial tests, the scintillation tubes were taped to the steel frame. However, most of the tubes were broken by solid debri from the strike plate. Therefore the scintillation tubes containing bacterial spores were placed inside wooden blocks to prevent shattering of the tubes after impact.

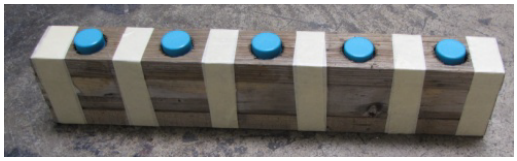


Figure 5. Wooden block the spore-containing tubes were positioned in. These wooden blocks with the spore-containing scintillation tubes were then placed in the spaces on the external periphery of the steel frame within the experimental chamber. The figure below displays a top view of the experimental chamber.

Figure 6. Top view of the steel drum experimental chamber with the wooden blocks containing scintillation tubes placed on the space outside the steel square frame but still within the chamber.

Many thermite experiment shots have been conducted; the most recent iodine pentoxide and aluminum thermite shot was selected as the representative experiment. In this particular experiment (shot number 1341), two spore-containing scintillation tubes were placed outside of the experimental chamber and served as the control tubes. Two other spore-containing scintillation tubes were placed within the experimental chamber and were exposed to the products generated from the impact-driven thermite reaction of iodine pentoxide and aluminum. The spore-containing tubes were subjected to an exposure time in the experimental chamber of one hour prior to rehydration and plating.

Recovery for post-experimental analysis

The recovery of spores after the impact-driven thermite reaction involved transferring of remaining spores from the scintillation tubes onto agar dishes for germination. This was achieved by rehydration of the scintillation tubes with

water, thorough vortexing, serial dilution, and plating on agar dishes. Different volumes of the same dilution were plated as parallels in order to increase statistical significance of the results. Control tubes were also placed outside the experimental chamber to determine efficiency of the recovery procedures.

Results and Discussion

Impact-driven reaction

In separate tests, projectiles were fired against the strike plate in an open range. High-speed videos of the impact were recorded. The following figure below displays a few selected time frames of the recorded impact video. Fireballs were generated from the impact-driven thermite reactions. Figure 7 shows a picture of the strike plate after impact. This demonstrates that the reaction can be ignited in the impact reaction.

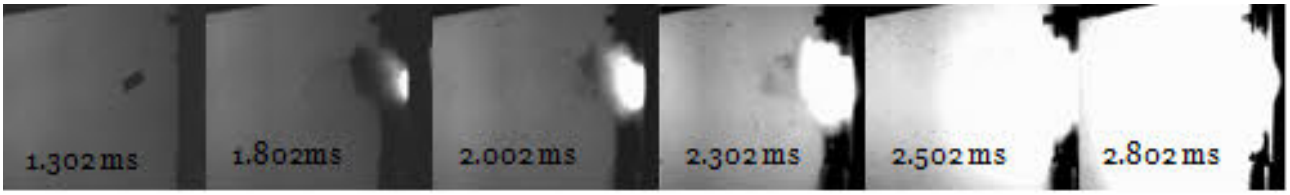


Figure 7. Selected time frames from high speed video of the impact-driven thermite reaction.

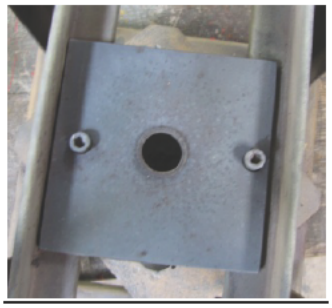


Figure 8. The steel strike plate after the impact-driven thermite reaction.

Scanning electron microscopy

Products collected from the impact-driven thermite reaction were observed under the scanning electron microscope. Energy-dispersive x-ray (EDX) spectroscopy was also conducted for chemical identification and elemental analysis of the samples obtained. Interesting surface features were observed.

"Cells" which seem to indicate the physical phenomena of crystallization and rapid solidification, were observed. The elemental analysis revealed the identity of these features as iodine with other traces of iron. The peak with the largest area under the curve was identified to be iodine.

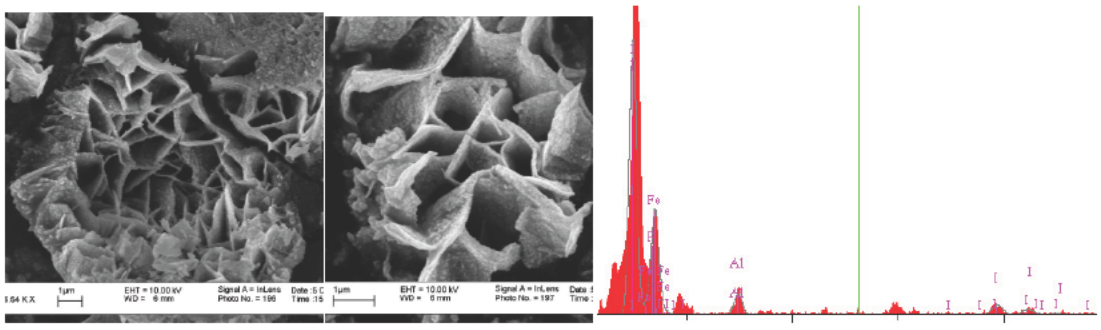


Figure 9. Scanning electron migrographs and EDX elemental analysis of iodine deposits after the impact-driven thermite reactions.

Rapidly quenched and alpha aluminum oxide was also observed as illustrated in the scanning electron micrographs below.

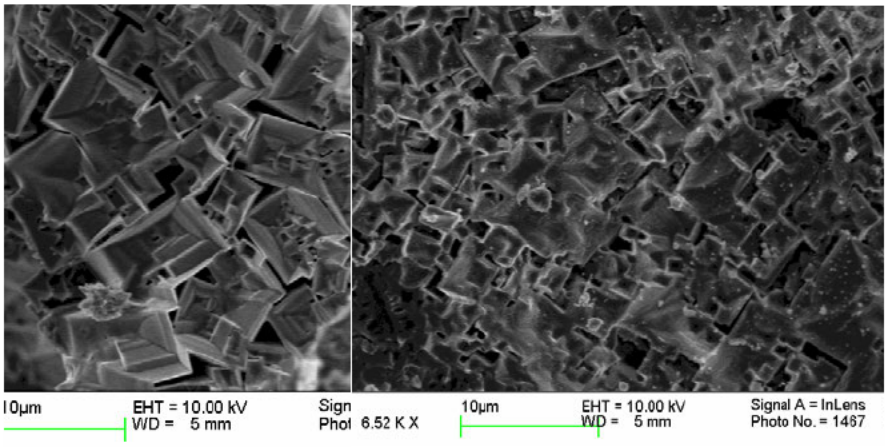


Figure 10. Platelets of alpha alumina were observed in post-impact products.

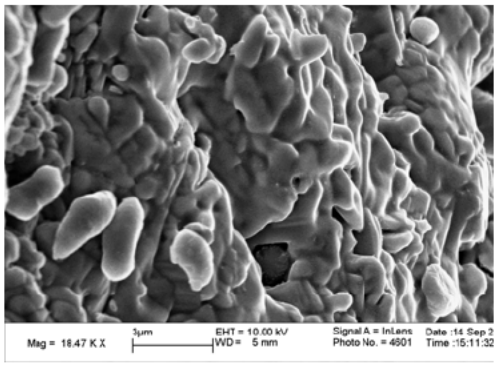


Figure 11. Alpha alumina particles recovered from the impact-driven tests.

In other tests, the reactions were initiated by a heat source. The morphology of the impact-driven reaction products was remarkably different from that of the combustion reactions. Combustion reaction particles exhibited amorphous spherical features instead.

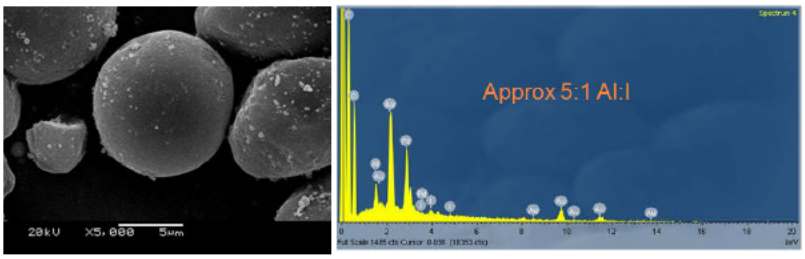


Figure 12. Scanning electron micrographs and EDX elemental analysis of recovered particles from iodine-aluminum thermite combustion experiments.

Control tubes

The number of colony forming units in the control tubes corresponded with the expected number of colonies from the serial dilution within the statistical scatter. Since there was approximately 100% germination, the colony forming units were normalized with respect to the theoretical population.

Chemical reactant exposure experiments

Germination results of bacterial spores co-plated with aluminum powder were identical to that of the control. The presence of aluminum powders did not appear to have any negative effect on the growth of *Bacillus subtilis* for both quantities of 10 mg and 100 mg.

However, there is a clear negative effect on the germination and growth of the bacterial spores in agar dishes plated with the iodine pentoxide aqueous mixture. Regions of brown discoloration on the agar dishes where the iodine pentoxide had been plated or diffused to had no growth as indicated below; all other areas of the agar plate contained lawn growth. It is suspected that iodic

acid—formed when iodine pentoxide is added to water— is the bacteriocidal agent that contributed to the inhibition of germination and growth.

Figure 13. Effect of 10 mg iodine pentoxide on germination of 10^6 *Bacillus subtilis* spores. The agar plate contained lawn growth except for regions where iodine pentoxide had been plated or diffused to.

Effect of impact-driven reaction on spores

The process of spore recovery from the scintillation tubes via rehydration was quite reliable. The two control tubes had an average of 90 ± 20 % recovery. Results are displayed below in Table 1. Figure 13 displays a typical picture of the agar plates with colony forming units. Theoretically, one countable colony arises from a single bacterial *spore*.

Table 1. Colony forming units of the recovered control tubes.

	50 µL plated (CFU)		*100 µL plated (CFU)*		
Control tube 1	152	58	199	239	279
Control tube 2	119	107	185	200	183
Expected	125		250		
Pooled average recovery	86 ± 20 %				

There were 2 spore-containing scintillation tubes that were exposed to the impact-driven thermite reaction conditions. Results are shown in tables 2 and 3.

Table 2. Colony forming units of the experimental tube 1.

	50 µL plated (CFU)			*100 µL plated (CFU)*	
Parallel 1	28	0		5	0
Parallel 2	2	2	1	2	6
Expected	125			250	
Pooled average survival	1.1 ± 0.6 %				

Table 3. Colony forming units of the experimental tube 2.

	50 µL plated (CFU)	*100 µL plated (CFU)*	
Parallel 1	5	5	6
Parallel 2	3	7	1
Expected	125	250	
Pooled average survival	2 ± 1 %		

No significant survival difference was noted between the two different tubes. This perhaps indicates that there is little effect of tube location on survivability. One tube had an average survival rate of 1.1 ± 0.6%, while the other tube had an average survival rate of 1.8 ± 1.2 %; these calculated values were based on the expected number of colony forming units. The pooled data from the

two tubes gives a survival rate of 3 ± 5%. Figure 14 shows representative plated results of the control tube and the tube that was exposed to the impact-driven thermite reaction from the experiment.

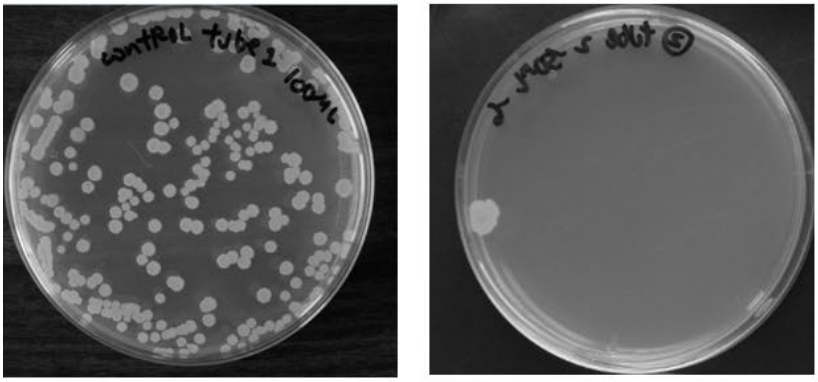


Figure 14. Plated results of control tube (left: 239 colonies) and tube exposed to the thermite reaction (right: 2 colonies).

The exterior of the experimental chamber was measured to be approximately 40 degrees Celsius. From this measurement, we may conclude that the temperature of the fireball generated within the experimental chamber must have only lasted a few seconds. We may therefore tentatively conclude that reaction products had a greater effect on the germination of bacterial spores than heat.

Future Directions

Despite the bacterial spore-killing rate of approximately 97%, however, the goal of the project is to investigate the experimental parameters for achieving

99.999 % killing rate. Different combinations of thermites are in the process of being tested, including a mixture of 62% iodine pentoxide and aluminum, 30% iodine pentoxide and neodymium, and 8% saran resin. Promising results have been obtained so far, with survival rates of 0.004 ± 0.005%. In addition, the chemical reaction products will be identified and analyzed; investigation of the biochemical mechanism of bacterial spore killing; temperature and pressure measurements via thermal sensitive paint and pressure gauges; aerosolization of bacterial spores.

Acknowledgements

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Thanks to Dr. Bless for introducing me to research and providing me with the amazing opportunity to work at IAT. He is whom I have learned about research and passion from. He is also the reason why I have considered incorporating research into my future career goal because I enjoy it so much. I have had the best P.I. and mentor any student can ask for and the impact he has had on me cannot be understated.

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Thanks to Dr. James Walker, Alexandra Blinkova for all of their guidance and support in microbiology—a huge component of this thesis work. Thanks to Harry Hart, Jay Frasch, Richard Eaves, and Bob Tucker for helping setup all the experiments.

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