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14. ABSTRACT

This research was a collaborative effort between experimental program at Auburn University and a computational modeling study at Pennsylvania State University. Determinations were made of the inactivation of aerosolized Bacillus Anthracis (Ba) sinlge spores exposed to temperatures from 165C to 275C for times between 25ms and 100ms. The data was used to anchor computational fluid dynamics (CFD) flow modeling of heat transfer into the areosolized Ba spores

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PROGRAM OVERVIEW

In this collaborative research, all experimental determinations of the thermal inactivation of Bacillus anthracis (Ba) spores were carried out at Auburn University and all computational modeling of the inactivation process was conducted at Pennsylvania State University. Emphasis was placed on providing the most exact values possible of thermal inactivation kinetics to the modeling team regardless of the limited application of the time-temperature range used to real-world problems.

EXPERIMENTAL

W.C. Neely

Methodology

Single-spore aerosol dispersions of Ba spores wereproduced by controlled injection into a nebulizer system. The apparatus and procedures were modified several times. In the final form the system consisted of these components:

Controlled volume injection system:

.

An electronically controlled syringe injector was used to inject Ba suspensions whose concentrations were near 10exp6 spores per m into an atomizer.

Atomizer:

The glass atomizer was set to produce droplets of ~1 nL volume. Thus, at spore concentrations of 10exp 6 per mL, each 1 nL droplet would contain ~ 1 spore,

Drying tube

A short glass drying tube heated to a temperature just above the boiling point of water was employed to vaporize the droplets containing the Ba spores. From the drying tube. the spore aerosol passed on into the exposure tube

Exposure tube

The exposure tube was of exactly known length and inner diameter. A typical length was 20 cm with a diameter of 1 cm. Other lengths and diameters were tested to assist in obtaining various residence times. Primarily however, the residence time of spores in the exposure tube was determined by the flow rate of the heated carrier gas,

Carrier gas

The carrier gas flow through a gas heating system was set using a mass flow control. The final volume of the heated gas was calculated from gas law equations and the effective flow rate thus obtained simply by dividing the tube volume by the flow rate.

Carrier gas heating

The carrier gas heating system was a quartz heat exchanger placed inside a tube furnace fitted with a temperature control system.

Spore collection

As the spores exited the exposure tube they impacted a cold surface so that the thermal inactivation process was immediately terminated. In this way, thermal exposure process was limited to the residence time of spores in the exposure tube.

Spore assay

The collected spores was removed from the cold surface by washing repeatedly with buffer solutions and their vaibility determined using standard techniques of serial dilution and plating onto growth media followed by incubation and colony counting. The Colony Forming Units (CFUs) so determined were taken to be the viable spores surviving the exposure.

Survival calculations

Although this setup can provide an exact knowledge of the number of spores injected into the exposure system, there was no practical method for accounting for the number lost to the walls of the apparatus or killed by physical impact. Accordingly, we used a relative survival calculation in which the number of survivors (CFUs) were referenced to the number of CFUs found for control runs with the exposure tube at room temperature. A set of control runs were made for each suspension each day.

Limitations

One drawback to the methods used in these experimental studies is the limitation of spore numbers to ~10^6 spores. This arises from the need to limit the concentration of injected spore suspension to ~10^6 spores per mL. With this concentration an average of one spore is dispersed in each nanoliter droplet from the atomizer. The total injection volume is limited to about 1 mL both because injecting significantly larger volumes would require more time than is available before the collection trap heats up and because the increased

concentration can lead to producing clumps of spores.. With these constraints we were limited in the extent of kill possible to measure and thus were able to study only a temperature range of 165 C to 275 C. and a time span of 25 to 100 ms. While this limits the direct value of these results to real-world application is quite acceptable for basic computation modeling use.

Experimental Results

Inactivation data

The percent survival at each of nine exposure temperatures, 438 K,461 K, 467 K, 473 K, 479 K, 486K, 491 K, 498 K, and 548 K, was determined for single-spore aerosols of Bacillus anthracis spores at exposure times of 25 ms, 50 ms, 75 ms, and 100 ms.

A minimum of 15 replicate runs for each time-temperature condition were used to generate each final data point . Standard deviations were obtained and are shown as error bars in the plotted data of Figure 1.

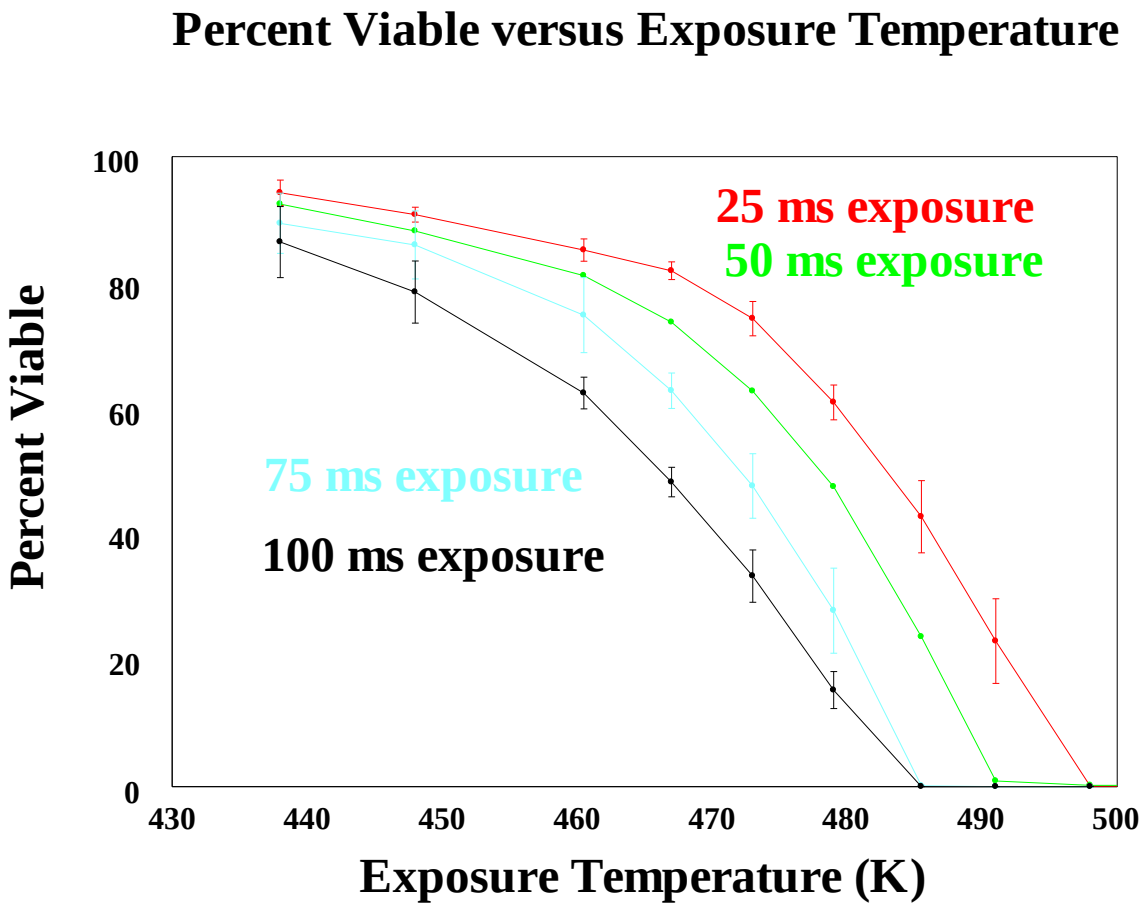


Figure 1. .Viable fraction of Ba spores following exposure to differing temperatures for varying times.

Experimental Conclusions

These data were obtained to provide anchoring for heat transfer modeling and do not encompass conditions most likely to be of significance in real-world weapon events. However, a few conclusions can be drawn from these results.

No dramatic difference was seen between exposures at the four different times tested. The curves were of similar shape and, as expected, were simply displaced from each other. This argues that there were no significant differences in the kinetics of inactivation processes in the time-temperature ranges tested.

More light is expected to be shed on this in current follow-on work where the biochemistry of the inactivation process(es) is of interest.

COMPUTATIONS

Deborah Levin

Fundamental Underpinning of DSMC

- The DSMC is a numerical method for solving the Boltzmann equation, under the assumption of
- a dilute, binary "gas": d << μ, d=diameter of particle, μ = mean spacing between particles = 1/n1/3 , n = number density;
- td << tc, td=mean duration of a collision, tc = mean time between a collision;
- molecular chaos or lack of correlation between two particles**.**

Technical Objectives and Approaches of Spore Heat Modeling

- Combine laboratory experiments with detailed modeling and simulation of spore-kill by heat transfer and by thermally enhanced chemical action

- Assess different spore kill mechanisms: heating, combustion, or corrosion

Modeling and simulation research challenges:

- Multiple length-scales: combine large-scale, continuum based modeling of laboratory and/or blast systems with spore near-field DSMC calculations.
- Two-phase flows,
- Chemically reacting, turbulent flow

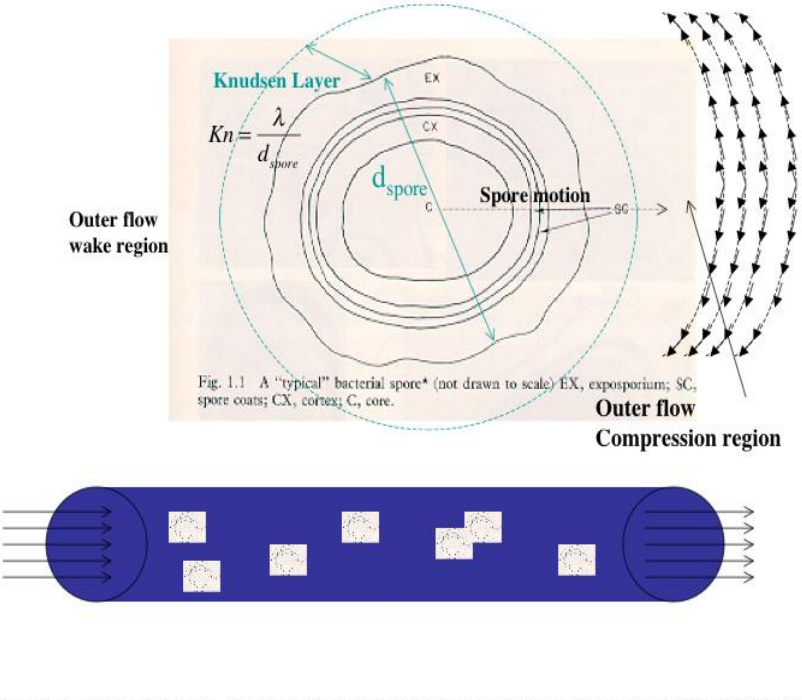


Fig. 1.1 A "typical" bacterial spore* (not drawn to scale) EX, exosporium; SC, spore coats; CX, cortex; C, core.

Figure 1.1 taken from Russell, A. D., *The Destruction of Bacterial Spores,* Academic Press, 1982, ISBN 0_12_604060_5.

Figure 2. Spore body in flow field

Computational Results

- Solution of the Navier-Stokes equations for the flow inside the device has been obtained by the GASP solver.
- 3rd order of accurate scheme was used,
- Flow was assumed to be incompressible and viscous.
- Working gas was Nitrogen.
- Flow assume to be laminar**.**

- The computational domain consists of two joint tubes which represent the inlet portion of the device an the main chamber.
- The boundary conditions were as follows:
- at the inlet: "Relax P_{Inflow}" which sets the velocity and extrapolates the pressure.
- at the wall: adiabatic or no energy exchange between the gas and the wall.
- at the exit: "P_{back}" which sets the exit pressure to a specific value and the other parameters were extrapolated.

Figure 1.3 shows the computational model with an enlargement of the entry section. Velocity profiles are color coded. This assumes no added turbulence from square inlet profiles, motionless mixers, or other means.

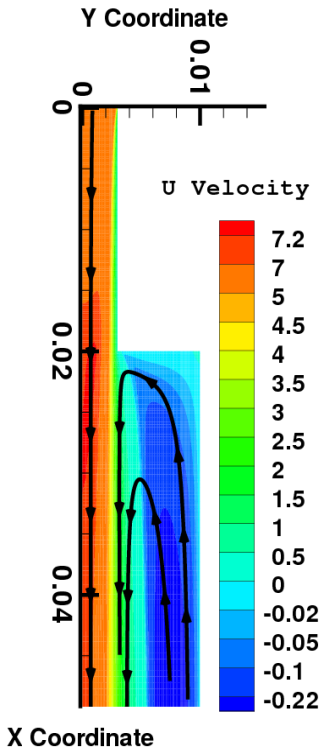


Figure 3 Velocity contours in the inlet

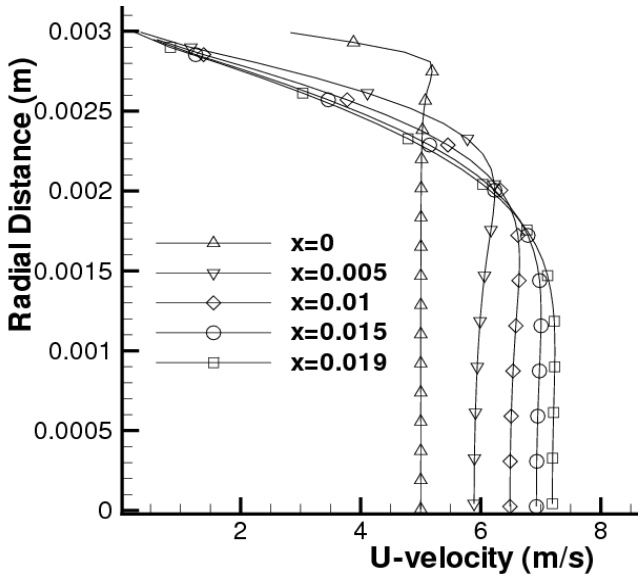
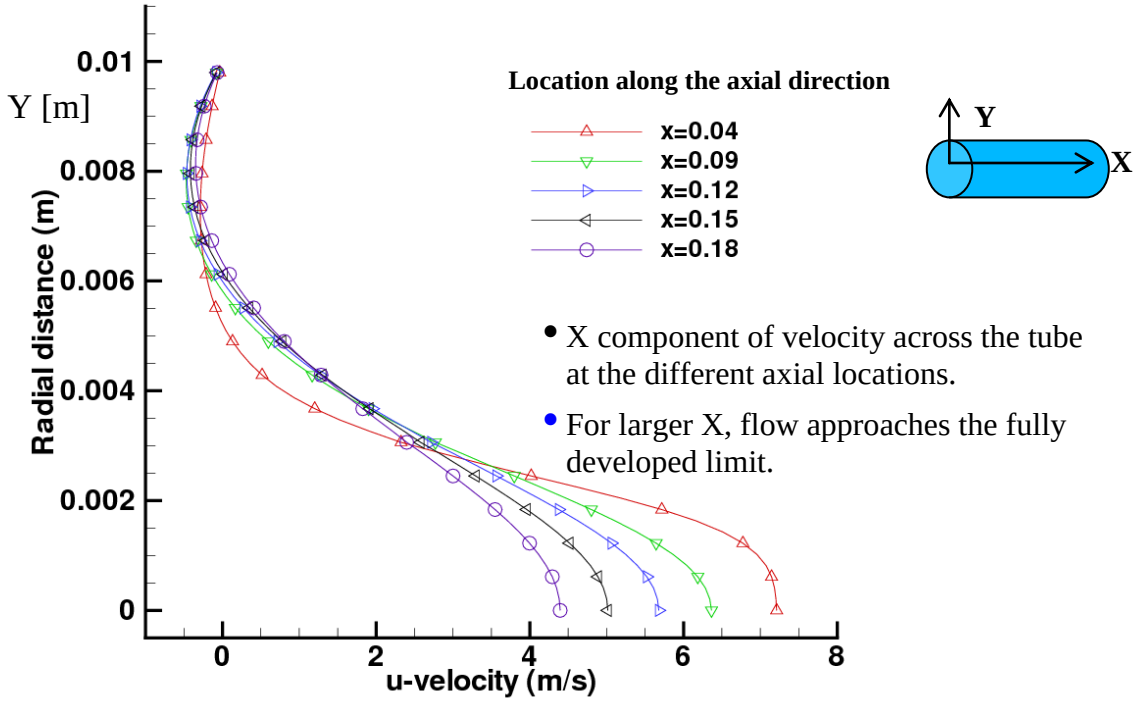


Figure.4

Note in Figure 4 that the flow accelerates in the inlet portion of the device due to decrease in the effective cross sectional area.

Streamwise Velocities Across the Second, Main Tube



- X component of velocity across the tube at the different axial locations.
- For larger X, flow approaches the fully developed limit.

Figure 5

The practical consequence of an extreme velocity profile arcross the tube is that particles (i.e. spores) travelling in the slower flow regions will be exposed for longer than calculated times and, conversely, those in the faster moving regions will be exposed for shorter times than calculated. The actual apparatus used to date has used a square-end inlet to induce turbulence and thus non laminar flow. Such a profile can, however, lead to eddy currents which may also provide excess exposure to spores caught in them. If this were to occur, the observed neutralization would be an upper limit. In laminar flow, the situation cannot be so well resolved.

Enlargement of Recirculation Area

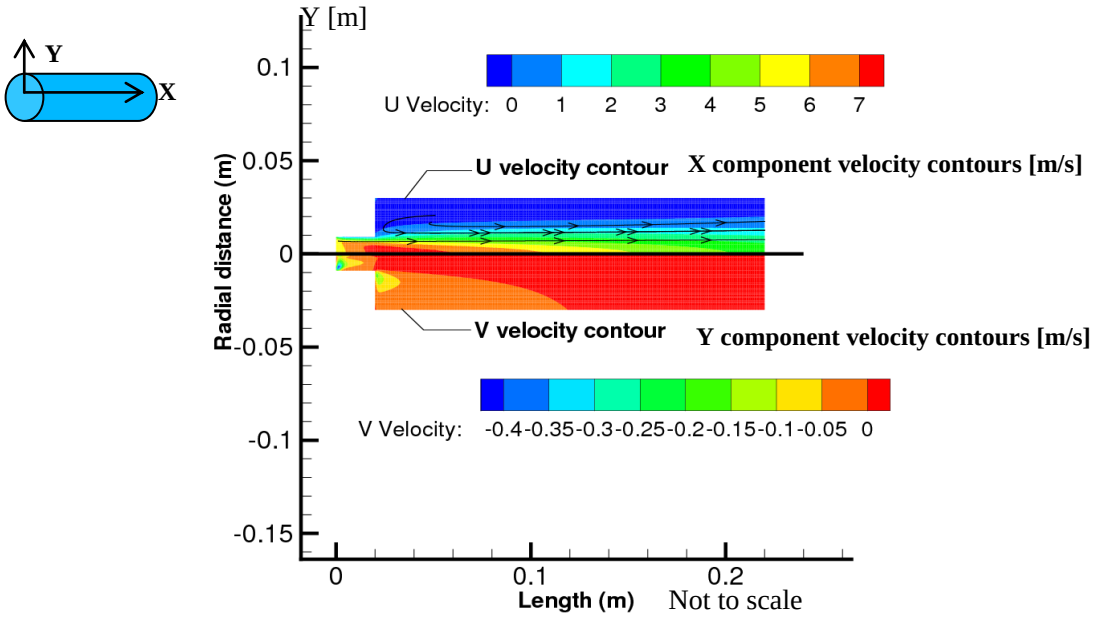


Figure 6

The recirculation area calculated for a graduated diameter change is illustrated. Computations for a square step change indicate the extent of eddy flows in the existing exposure system.

Thermo-Structural Response Modeling

Here we summarize the research aspects related to the coupled thermo-structural response. More details can be found in our conference paper [**"**Thermo-structural Studies on Spores Subjected to High Temperature Gas Environments," AIAA Paper No. 009-3752, submitted to the Journal of Thermophysics and Heat Transfer].

The typical structure of a spore is shown in Fig. 2 below. The operative length scale is on the order of micron with a diameter ~ 2 μm, a cylindrical/spherical shape, and the structural integrity is provided by cortex or PG. The threat from dangerous spores such as Bacillus anthracis "anthrax" is well known. Though modeling and simulation,

validated by experiment, we studied ways to minimize the damage caused by the release of such spores by deactivating/annihilating them.

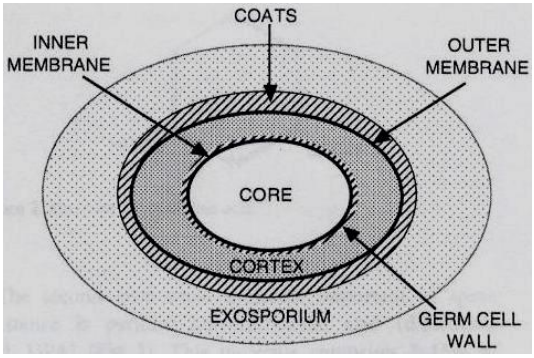


Figure 7. Typical Spore Structure

There are large uncertainties in the mechanical properties of spore and these properties also vary significantly with the relative humidity of the spore wall. Spores are also known to have a wide distribution in stress (including heat) resistance and differences arise because of the sensitivity culture conditions under which spores are grown. The table below shows the variation in two of the important structures properties of spores (estimated from Ref. [i]), rubber, steel, and other related materials. In the first step of our coupled thermal-structural analysis we examined a spore failure mode without considering simultaneous spore deformation. The spore was assumed to have composite water and spore wall properties. When the spore is exposed to a high temperature gas, the expansion of the assumed 1% of water exerts pressure on its walls, but in the first set of calculations the spore was assumed to remain rigid. Realistically, however, the spore could break or develop cracks at a specified level of internal pressure, depending on its stress resistance capability. Pressure values were obtained from the NIST tables and for the exposure tube conditions were typically in the super heated regime. Figure 2 (LHS) shows the calculated spore internal pressure, which is unrealistic because spores are not expected to withstand such high pressures and should deform to reduce the stress levels. It also shows that the use of the ideal gas properties would significantly under predict the spore internal pressure.

Material	Modulus of Elasticity (GPa)	Tensile Strength (MPa)
Spore wall	20	300
Rubber	0.01-0.1	5-10
Steel	200	440
Carbon Nano Tube	1000	63000
Red Blood cell	~0.001	---

Table 1. Typical Material Properties

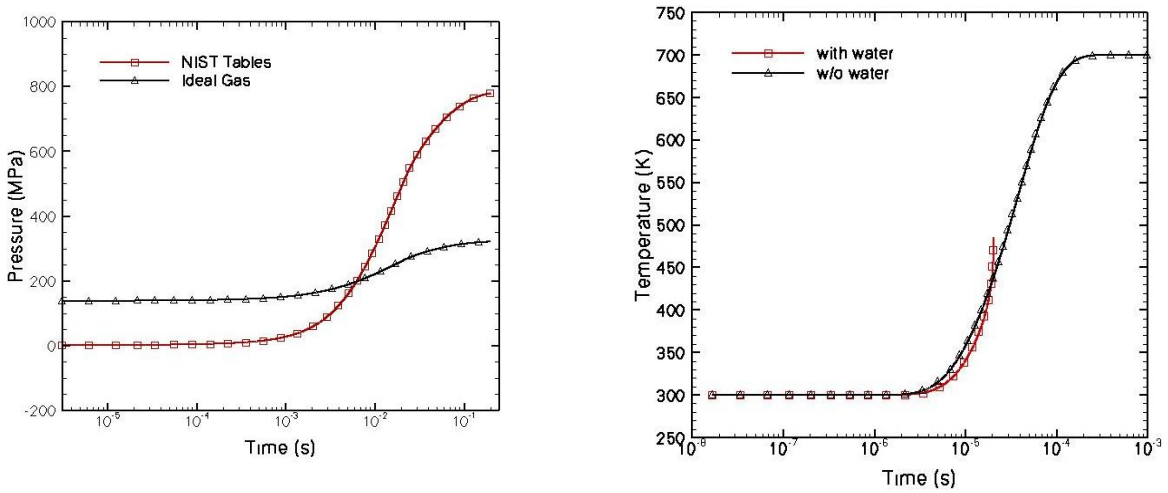


Figure 8. (LHS) Calculated spore internal pressure vs. time assuming a rigid spore for a final spore temperature of 700 K. (RHS) Calculated temperature increase for a spore without and with a 1% concentration of water. The red curve shows the result of the dynamic thermo-structural response considering inertia affects.

In terms of the second approach used in the coupled thermo-structural analysis, the spore is assumed to be dynamic. Even with the inclusion of spore deformation, however, there are two possible approaches, with and without inclusion of inertia effects. The latter accounts for the change in material properties as a function of increase in membrane diameter. The work of Wineman et al[ii] shows that failure to include inertial effect results in unrealistic results. The inclusion of initial effects provides a relationship between the internal pressure and the spore radius of,

$$p - \frac{2\sigma t_h}{r} = \rho t_h \frac{d^2 r}{dt^2};$$

where the above equation is obtained from the stress-strain characteristics of a spore and ρ is the density of the membrane material, σ is the stress state the elastomeric material is

subjected to, p in the internal pressure, and t_h and r are the spherical membrane thickness and radius, at time t, respectively. The above equation can be discretized and solved using an explicit finite difference procedure. Since this equation involves the internal pressure, it couples the spore heat transfer and thermal response to the spore's structural response. The spore radius is updated using the above equation and with the updated spore radius the strain is computed using,

$$\frac{\sigma}{\Delta r / r} = E$$

where E is the strain. The stress is estimated from the strain and is then used for computing the time varying thermo-structural response of the spore. The process is repeated until the allowable limit of stress is exceeded or until a time has passed such that the spore would have left the exposure tube system.

Unfortunately the stress-strain characteristics for spore walls could not be found, and instead, scaled-up characteristics of rubber were used. Using a tensile strength of 300 MPa for the spore wall composed of PG (see Table 1), it was found that the stress exerted by the internal pressure causes the spore wall to break or fail at 490 K for a gas at a temperature of 700 K (see Fig. 9, RHS). Consideration of this spore failure mechanism suggests that the spore will not reach an internal temperature of 700 K because it will explode first.

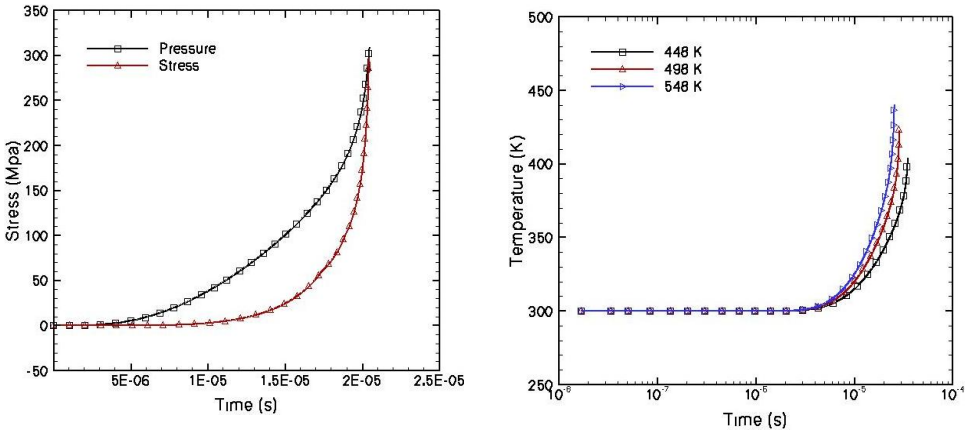


Figure 9 Thermo-structural response to different operating gas temperature conditions. (LHS) Comparison of pressure and stress as a function of time through spore breaking point of 300 MPa. (RHS) Temperature profiles obtained from the coupled analysis for three exposure tube operating conditions.

Figure 9 shows the time variation of the spore internal pressure (1% water) and stress as well as the temperature profiles for three gas temperature operating conditions of the exposure tube predicted by the coupled thermo-structural response model. The results show that for the operating temperatures in the range of 450 to 550 K the spore explosion mechanism is a very possible failure mode. Figure 9 shows preliminary exposure tube data for spore survivability. It can be seen from the figure that the survivability drops

from 100% at 400 K to 0 at 500 K, indicating a temperature tolerance limit for spore. Within the error bars, the change of spore residence time from 50 to 60 ms does not change the survivability results. Examination of the total heat content for the three exposure tube temperatures shown in Fig. 3 (i.e., 448, 498, and 548 K) for a spore at steady state of 336, 373.5, and 411 MJ/m^3 suggests that spore lethality sensitivity to total heat absorption alone is not sufficient to explain the exposure tube data. Note that the thermal response of spore conglomerates shows a large difference as compared to single spore and the may be responsible for the observed survivability variation with temperature and residence time. This and other uncertainties in the spore thermal and structural properties will be examined in future research.

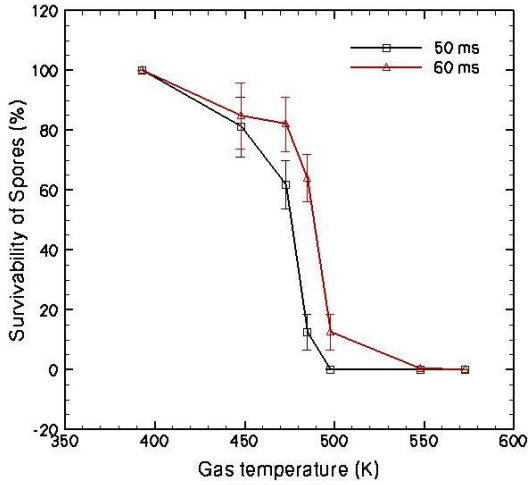


Figure 10 Exposure tube data of spore survivability.

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[i] Thwaites, J. and Mendelson, N., "Mechanical Properties of Peptidoglycan as Determined From Bacterial Threat," *Int J. Biol. Macromol*., Vol. 11, 1989, pp. 201-206.
[ii] Wineman, A., "Dynamic Inflation of Elastomeric Spherical Membranes undergoing Time Dependent Chemorheological Changes in Microstructure", International Journal of Engineering Science, 2008.