Heat Inactivation of Embedded Bacterial Spores

Berenece Badillo1, Wayne Schubert2

1 California Polytechnic University Pomona
2 Biotechnology and Planetary Protection, Jet Propulsion Laboratory, California Institute of Technology

Abstract

Bacterial spores embedded in a polymeric matrix may be up to 10 times more resilient than spores found on the surface of a material. The resiliency of these spores could result in a lower than expected microbial reduction using current standards. The goals of planetary protection are to measure, control, and reduce spacecraft microbial contamination in an attempt to minimize the chance of contaminating other worlds with Earth lifeforms or vice versa. The primary method used to microbially reduce spacecraft materials is heat microbial reduction, or HMR. In order to determine the minimum effective times and temperatures to inactivate particularly resistant bacteria, D-values obtained from indicator organism survivor plots are calculated. These D-values represent the time required to reduce the microbial population by 90%. Bacillus atrophaeus, a highly resistant spore forming bacterium, was embedded in 3M 2216 Scotch weld epoxy, a spacecraft material. In our experiments, samples of epoxy embedded with bacterial spores were heated at chosen temperatures for specified times, cryogenically ground, diluted, poured plated, and survivors counted. Preliminary results gave D-values of 12.6 hours at 125°C and 0.561 hours at 150°C. Given these results, further study is necessary to confirm these higher D-values than the current NASA spec.

Objective

This study is a revisit on previous experiments with Scotch 3M 2216 epoxy and the target organism, Bacillus atrophaeus. Specifically lower temperatures will be explored to further understand the behavior of bacterial spores embedded in this particular epoxy.

Methods

B. atrophaeus spores were embedded in Scotch 3M 2216 epoxy. After curing the samples in 2 ml centrifuge tubes they are exposed to various temperatures for different times in a silicone oil bath. Afterwards, the samples are cryogenically ground, diluted, and sonicated to release any surviving spores. After performing a series of serial dilutions, sample dilutions 10^2 through 10^8 are poured plated using standard TSA agar in quadruplicate. Any surviving organisms were counted and the colony forming units per gram were calculated.

Results

![Figure 1: Flow chart of the procedure starting from spore embedding through colony counting.](image)

![Figure 2: The survivor plot at 125°C of the current data gives a D Value of 55.6 minutes and the survivor plot of the previous data gives a D Value of 588.2 minutes.](image)

![Figure 3: The survivor plot at 150°C of the current data gives a D Value of 61.32 minutes and the survivor plot of the previous data gives a D Value of 43.38 minutes.](image)

![Figure 4: The survivor plot at 104°C gives a D Value of 10,000 minutes.](image)

![Figure 5: Unaveraged Arrhenius plots for previous and current data using normal time. The two previous points that were problematic were the previous data points for 104°C and 151°C.](image)

![Figure 6: The survivor plot at all temperatures plotted together on one graph to summarize all the current data.](image)

![Figure 7: The survivor plot at 104°C gives a D Value of 10,000 minutes.](image)

![Figure 8: The survivor plot at 125°C gives a D Value of 55.6 minutes and the survivor plot of the previous data gives a D Value of 588.2 minutes.](image)

![Figure 9: The survivor plot at 150°C gives a D Value of 61.32 minutes and the survivor plot of the previous data gives a D Value of 43.38 minutes.](image)

![Figure 10: The survivor plot at 104°C gives a D Value of 10,000 minutes.](image)

![Figure 11: The survivor plot at 125°C gives a D Value of 55.6 minutes and the survivor plot of the previous data gives a D Value of 588.2 minutes.](image)

![Figure 12: The survivor plot at 150°C gives a D Value of 61.32 minutes and the survivor plot of the previous data gives a D Value of 43.38 minutes.](image)

![Figure 13: The survivor plot at 104°C gives a D Value of 10,000 minutes.](image)

![Figure 14: The survivor plot at 125°C gives a D Value of 55.6 minutes and the survivor plot of the previous data gives a D Value of 588.2 minutes.](image)

![Figure 15: The survivor plot at 150°C gives a D Value of 61.32 minutes and the survivor plot of the previous data gives a D Value of 43.38 minutes.](image)

![Figure 16: The survivor plot at 104°C gives a D Value of 10,000 minutes.](image)

Data Analysis

Three points were generated from each time point. These values were averaged and normalized to the control. The logarithm of this was then plotted against time for each temperature. To obtain a D-value, the inverse of the slope of the resulting line was taken. The natural log of each lethality rate constant was taken and plotted against the inverse temperature in Kelvin to produce an Arrhenius plot. The slope of the resulting line, B, was then used to calculate the equivalent time using the following formula:

$$E_{q} = \frac{1}{B}$$

where $E_{q}$ is the calibrated energy and $B$ is the Arrhenius pre exponential factor.

Conclusion

This experiment was a repeat of a previous experiment done in order to corroborate the data and to explore the behavior of embedded spores heated to a lower temperature. The resulting Arrhenius plot of the current data compared to the previous data corrects for certain dubious points. The D-values obtained from the current data compared to the previous data differ greatly at 115°C. The D-values at 125°C are more similar and the points at 150°C and 170°C. Engineers have traditionally proposed 104°C as a heating temperature. This temperature range has no recent previous data to compare to therefore, it was an interesting data point to explore. The longest nominal time heated to this temperature was for 1 week, or 168 hours. Heating this long however, still did not reach a one-log reduction in bioburden. When all survivor data are plotted together, the 104°C temperature data dwarfs all the other survivor data. Repeating experiments are important for planetary protection in order to increase confidence in the procedures proposed. The repeated data falls under the same order of magnitude as previous experiments, further corroborating the procedures proposed. This data also suggests that heating at lower temperatures for long times isn’t as effective at reducing bioburden than heating at high temperatures for shorter amounts of time.

Acknowledgements

This research was carried out at the Jet Propulsion Laboratory, California Institute of Technology, under a contract with the National Aeronautics and Space Administration. This work could not have been possible without the help of Wayne Schubert at the Jet Propulsion Laboratory and the rest of the Biotechnology and Planetary Protection team. Funding was provided by the STEM Teacher and Researcher Program and facilitated by both Petra Kneissl from the Education Office at the Jet Propulsion Laboratory and Carol Casey from the Student Faculty Program Office at the California Institute of Technology.