

Title: Examination of *B. subtilis* var. *niger* spore killing by dry heat methods
Michael J. Kempf and Larry Kirschner
Biotechnology and Planetary Protection Group, Jet Propulsion Laboratory, California
Institute of Technology, Pasadena, CA 91109

Dry heat microbial reduction is the only NASA approved sterilization method to reduce the microbial bioburden on space-flight hardware prior to launch. Reduction of the microbial bioburden on spacecraft is necessary to meet planetary protection requirements specific for the mission. Microbial bioburden reduction also occurs if a spacecraft enters a planetary atmosphere (e.g., Mars) and is heated due to frictional forces. Temperatures reached during atmospheric entry events ($>200^{\circ}\text{C}$) are sufficient to damage or destroy flight hardware and also kill microbial spores that reside on the in-bound spacecraft. The goal of this research is to determine the survival rates of bacterial spores when they are subjected to conditions similar to those the spacecraft would encounter (i.e., temperature, pressure, etc.). *B. subtilis* var. *niger* spore coupons were exposed to a range of temperatures from 125°C to 200°C in a vacuum oven (at <1 Torr). After the exposures, the spores were removed by sonication, dilutions were made, and the spores were plated using the pour plate method with tryptic soy agar. After 3 days incubation at 32°C , the number of colony-forming units was counted. Lethality rate constants and D-values were calculated at each temperature. The calculated D-values were: 24 minutes (at 125°C), 9 minutes (at 135°C), and <0.1 minutes (at 150°C). The 125°C and 135°C survivor curves appeared as concave-downward curves. The 150°C survivor curve appeared as a straight-line. Due to the prolonged ramp-up time to the exposure conditions, spore killing during the ramp-up resulted in insufficient data to draw curves for exposures at 160°C , 175°C , and 200°C . Exploratory experiments using novel techniques, with short ramp times, for performing high temperature exposures were also examined. Several of these techniques, such as vacuum furnaces, thermal spore exposure vessels, and laser heating of the coupons, will be discussed.