# OPTIMIZING THERMAL AND RADIATION EFFECTS FOR BACTERIAL INACTIVATION*

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**Abstract.** The temperatures required for dry-heat spacecraft sterilization have been known to degrade heat-sensitive components. Thermoradiation, the simultaneous application of dry heat and gamma radiation, can provide the same degree of microbial inactivation as dry heat alone while substantially reducing component degradation. This is made possible by the synergistic effects produced when relatively low levels of these agents (e.g., 90 to 350 krads and 60° to 105 °C) are applied simultaneously, thus permitting the use of lower temperatures and a reduced duration of heat exposure. The effects of temperature, radiation dose rate, and relative humidity on microbial inactivation during thermoradiation exposure have been established.

## 1. Introduction

Since space exploration plans were first formulated, it has been recognized that microorganisms carried on spacecraft could contaminate other planets, with a resulting loss of unique and invaluable scientific information (Lederberg, 1960). These considerations have led to the formulation of recommendations concerning planetary quarantine and spacecraft sterilization by the Committee on Space Research (COSPAR) of the International Council of Scientific Unions. U.S. policy guidelines based on COSPAR resolutions currently require complete heat sterilization of the entire lander system to assure a probability of contamination of less than $10^{-3}$ during the entire unmanned planetary exploration of a planet (COSPAR, 1969).

The effects of such dry heat sterilization on the reliability of some components have presented many problems to both U.S. and Soviet engineers. Although it is not clear at this time what methods the Soviets intend to employ, their procedure outlined at COSPAR (Astofyeva, *et al.*, 1966) included the various techniques of dry heat, gamma radiation, filtration, and chemical sterilization.

From the variety of sterilizing agents proposed by the Soviets, and from our own experience in the U.S., it is apparent that no single sterilization agent presents a completely satisfactory solution to spacecraft sterilization. Dry heat alone requires temperature levels that affect the reliability of some materials and components. Ionizing radiation in the 2.5- to 5-million-rad ($H_2O$) range also causes degradation and change in physical properties. And, finally, the gaseous or chemical agents such as phenols, formaldehydes, and ethylene oxide are effective on surfaces but less effective on contamination buried in solid materials. For these reasons and from evidence from other studies (Graikoski, 1961; Koesterer, 1964), we have been investigating combinations

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of these agents in which synergistic effects may permit sterilization at reduced temperatures and acceptable levels of radiation. This paper describes part of a continuing research activity directed toward optimizing thermoradiation by achieving an insight into the synergistic mechanisms associated with bacterial inactivation and developing predictive mathematical models to depict these relationships.

## 2. Methods

*Bacillus subtilis* var. *niger* spores from the Fort Detrick stock was used in both the thermoradiation and comparative dry heat experiments. After removal of vegetative material by multiple centrifugation, the spore stock was suspended in a 95 percent ethanol solution in concentrations of $10^7$ and $10^9$ spores per ml, and stored at 4°C until used.

Test samples were prepared by pipeting $10^6$ spores on aluminum foil discs. A second disc formed a cover, and four disc sets or replicate samples were retained between two aluminum strips wired together. This method of assembly permitted the samples to be hung vertically in an oven and prevented loss or damage during normal handling. Simultaneous heat and radiation exposure was achieved by placing the sample assemblies in a recirculating oven inside the Sandia Gamma Irradiation Facility (GIF). The desired dose rate was attained by locating the oven an appropriate distance from the 16-kilocurie cobalt-60 source. Silver phosphate or cobalt glass dosimeters were placed on sample strips to verify the computed dose rate. All temperature and humidity recorders, the controlled humidity system (Garst, 1970), and the radiation source controls were outside the GIF cell, with necessary connections through the cell wall.

After exposure to the heat and/or radiation environment, each sample was removed from the strip and insonated for two minutes in a 50 ml beaker containing 10 ml of sterile 0.1% Tween 80 water. Ten-fold serial dilutions were made as required. Dilutions were plated in duplicate on Trypticase Soy Agar underlay and then overlayed with the same media. The plates were incubated at 35°C and counted after 72 hours. All microbiological preparation and recovery procedures were performed in a Class 100 laminar downflow clean room (Reynolds, 1969).

For experiments that were conducted to determine the effectiveness of thermoradiation on bacterial spores encapsulated in solid materials, *B. subtilis* var. *niger* spores were mixed with methylmethacrylate monomer and polymerized into thin sheets. Samples containing $1.2 \times 10^5$ spores were cut from the sheets, taped to aluminum strips, and exposed to gamma radiation in the same manner described for surface contamination. To recover the buried organisms, we dissolved the chips in acetone, cleaned and filtered the spores, and plated the filters on Trypticase Soy Agar.

## 3. Results and Discussion

A series of experiments was performed to compare the sterilization effectiveness of dry heat alone, ionizing radiation alone, and then the simultaneous application of dry

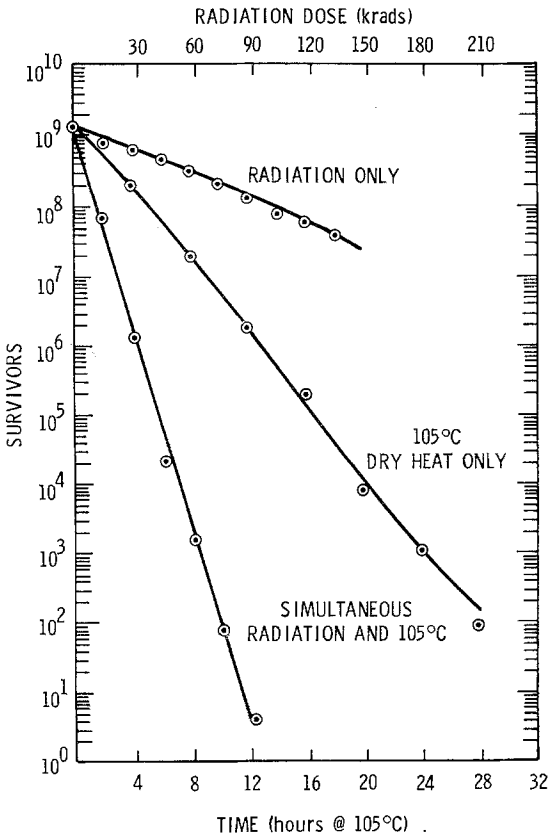


Fig. 1. A comparison of the inactivation of *Bacillus subtilis* var. *niger* using gamma radiation at room temperature, dry heat alone at 105°C, and then simultaneous dry heat and gamma radiation.

heat and ionizing radiation at temperature levels of 125°C, 105°C, and 100°C. Figure 1, typical of these experiments, compares the inactivation of these sterilization agents singly, then in combination. For example, gamma radiation alone will reduce the population approximately 1 log for each 100 Krad ($H_2O$) dose applied. For dry heat alone, an 8-log reduction in population requires 36 hours at 105°C. Thermoradiation, the application of heat and gamma radiation simultaneously, reduces the population 8 logs in 12 hours or $\frac{1}{3}$ of the time required for dry heat. The lethality attributed to radiation alone in this 12-hour period would be approximately 1 log and, for dry heat alone, about 3 logs. Thus, half of the total reduction in population, 4 logs, is due to synergism in the simultaneous application of heat and radiation. Additional experiments have revealed that this synergistic relationship of heat and radiation exists at a temperature as low as 60°C. Thermoradiation *D* values * at a dose rate of 8 krads/hour,

* A *D* value is the time required for a given microbial population to be reduced by 90% or 1 log in population in a given temperature. The *D* value can also be the radiation dose required to reduce a given population by 1 log.

varied from 1.5 hours at 105°C to 3 hours at 90°C and 6 hours at 60°C (Reynolds, 1970). These *D* values represent a rather significant reduction in time required for sterilization. For example, the dry-heat *D* values for *B. subtilis* var. *niger* at 60°C will range from 53 to 274 hours (Vesley *et al.*, 1969), depending on the moisture condition of the spores.

An evaluation of dose-rate sensitivity was also made to permit optimization of radiation levels at a given temperature. This would permit sterilization of spacecraft with a minimum environmental stress to components and materials. A series of experiments at 105°C with dose rates of 2.5, 5, 13, 22, 36 and 50 krads/hour is sum-

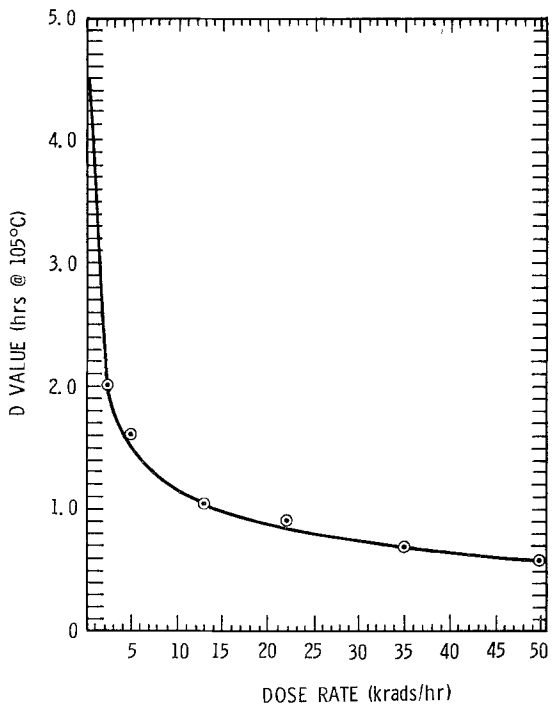


Fig. 2. Thermoradiation *D* values as a function of dose rate for the inactivation of *Bacillus subtilis* var. *niger* spores.

marized in Figure 2. Beginning with 4.5 hours for dry heat alone, there is a very rapid reduction in *D* value as gamma-radiation dose rates are increased to about 15 krads/hour, where higher dose rates yield marginal reduction in *D* values. This does, however, afford the flexibility of dose rate/total dose selection for materials or components of differing radiation sensitivity. For example, a typical optimum dose rate of 12 krads/hour at 105°C would result in a *D* value of 1.1 hours. Based on a 10-log population reduction for sterilization of a given spacecraft, the time for sterilization would be 11 hours, with a total gamma dose of 132 krads. For systems of lower radiation sensitivity, a higher dose rate of 36 krads/hour would yield a *D* value of 0.7 hour, a

sterilization time of 7 hours, and a total dose of 252 krads. This is a substantial reduction from the normal (Bruch, 1966) 50 hours required to sterilize by dry heat alone at 105°C.

Sensitivity of thermoradiation to additional parameters has also been investigated. An increase in moisture content of bacterial spores as a result of changes in laboratory relative humidity from 20% to 60% RH caused a two fold increase in the dry-heat $D$ value. Thermoradiation experiments performed under the same changes in humidity, however, exhibited no change in $D$ value.

Another aspect of spacecraft sterilization that has created considerable concern is the greater heat resistance of bacterial spores when they are encapsulated in solid materials. Angelotti *et al.* (1968) found a thirty-fold increase in $D$ values for *B. subtilis* var. *niger* when comparing sterilization of spores on surfaces with spores in a lucite or epoxy matrix. Since the encapsulated contamination is the most difficult to sterilize, we have tested the thermoradiation technique on spores in methylmethacrylate. These experiments performed at 105°C demonstrated the effectiveness of thermoradiation on buried contamination. $D$ values were determined to be 5 hours at a dose rate of 12 krads/hour. This is $\frac{1}{6}$ of the time required for heat alone at that same temperature.

## 4. Summary

Using the NASA standard test organism, *B. subtilis* var. *niger*, and with limited tests on other organisms, we have found that the synergistic effects produced by simultaneous low-level heat and radiation permit sterilization in a fraction of the time required when sterilants are applied singly. As a consequence of this technique, the lower stress applied to spacecraft could significantly improve the reliability of components and materials in future missions. Further, these low levels of exposure suggest potential applications in foods, pharmaceuticals, cosmetics, and medical products, but, in particular, spacecraft sterilization.

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