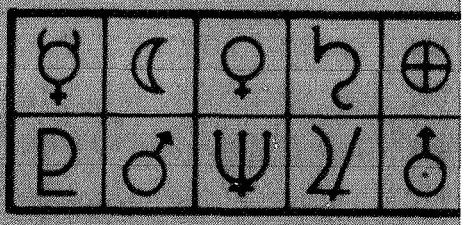
Research Report



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June 1970

A STUDY OF THE EFFECTIVENESS OF THERMORADIATION STERILIZATION

Marcel C. Reynolds
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ABSTRACT

Temperatures required for thermal sterilization are known to degrade certain heat sensitive components, materials and products. Simultaneous application of lower temperatures and low levels of gamma radiation produces a synergistic effect which can sterilize with fewer damaging side effects. A means of determining the optimum balance between heat and gamma radiation is demonstrated.

*This work was conducted under Contract No. W-12,853, Bioscience Division, Office of Space Science and Application, NASA Headquarters, Washington, D.C.

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A STUDY OF THE EFFECTIVENESS OF THERMORADIATION STERILIZATION

Introduction

The degradation of certain spacecraft hardware during dry heat sterilization has presented many problems to NASA, its contractors and suppliers. Alternate methods of achieving effective sterilization without significant degradation are being investigated.

This report presents recent data in the continued study of thermoradiation effects. The data presented in SC-RR-69-857, "The Feasibility of Thermoradiation for Sterilization of Spacecraft - A Preliminary Report," established a synergistic effect between dry heat and gamma radiation over a range of temperature and moisture conditions. This subsequent report expands the lower temperature range of investigation down to 60°C. In conjunction with the lower temperatures, an effort has been made to maximize the complementary effects of heat and gamma radiation by determining the most efficient balance between the thermal and radiation levels. Using these most efficient combinations, we would hope then to sterilize with the minimum environmental stress applied to components, materials and products. This would tend to minimize the side effects that can result from sterilization. Typical of these effects are creep, tensile strength change, brittleness, discoloration, change in electrical characteristics, loss of potency in drugs, and off flavors in food.

Surface Contamination Studies

This phase of the study pertains to the microbial samples deposited on the surface of a substrate and exposed to the desired environments.

Materials and Methods

Preparation of Samples--The organism used for the experiments was Bacillus subtilis var. niger of the Fort Detrick stock. The spore stock was cleaned of vegetative material and suspended in 95-percent ethanol to a concentration of 10^7 per ml. The suspension was maintained at $4^{o}C$ during storage.

Prior to use, the spore suspension was insonated for two minutes to disperse the spores within the ethanol. The samples were prepared by pipeting 0.1 ml of the suspension onto the surface of aluminum foil discs. The discs were 1.25-inch diameter cut from biological grade aluminum foil 0.0015-inch thick. The samples were then allowed to air dry until all of the ethanol had evaporated. When dry, the inoculated discs were assembled on aluminum strips, 1.50-inches wide by 0.020-inch thick. Four sample discs were placed on each strip, a single clean foil disc was placed over each sample and then another aluminum strip placed on top and held in place with wire clamps. This assembly of the discs clamped between two strips permitted considerable handling and suspending the assembly in a vertical position without loss or damage to the sample discs. The assembled sample strips were then placed in a desiccator over Drierite in a vacuum for 15 hours prior to exposure to the sterilization environment. All of the inoculation and assembly operations were performed in a Class 100 laminar airflow clean room.

Exposure Methods--The thermal environment was provided by a recirculating air temperature chamber having a volume of .578 ft^3 with a rail arrangement in the door to hold the aluminum strips. The temperature was controlled and recorded to an accuracy of $\pm$ $0.5^{o}C$. The radiation environment was provided by the Sandia Gamma Irradiation Facility (GIF). This facility contains remote handling equipment to introduce and remove the source and includes visual, physical, and electrical access with necessary safety controls. The cobalt-60 source is introduced in a corner of the cell, which is 7 feet by 8 feet by 8.5 feet high. The dose rates range from 1×10^6 rads/hr to 4×10^3 rads/hr depending on the location of the sample within the cell.

Moisture content of the air in the temperature chamber was controlled by a system[1] of mixing dry air from a desiccant bed with moist air from a saturator to a specific level and introducing it into the chamber at a rate of about 1 cfm. The relative humidity (RH) was measured and recorded with lithium chloride sensors to an accuracy of ±1 percent at room temperature. Relative humidity measurements were taken both at the input to the mixing chamber and from an air sample withdrawn from the temperature chamber and cooled to room temperature. The input air was adjusted to provide the desired level of RH as measured in the output air sample.

For each experiment, the temperature chamber was placed in the GIF cell at the appropriate distance from the cobalt-60 source, for the desired dose rate. The chamber was positioned so the sample strips assembled with the foil discs were vertical and the face of the strips was perpendicular to the direction of the gamma rays. The temperature chamber controller and temperature recorder were located outside the cell with necessary cable connections passed through the cell wall. The humidity control system was also located outside the cell. The input air to the temperature chamber was introduced through a 3/8-inch copper tube and the output air sample was withdrawn through a 1/4-inch copper tube. Both tubes passed through the cell wall. A block diagram of the equipment set-up is shown in Figure 1. Silver phosphate or cobalt glass dosimeters, depending on the dose, were placed on selected sample strips to verify the computed dose rates.

Recovery Methods--Each sample strip when removed from the temperature chamber was wrapped in sterile aluminum foil and returned to the clean room facilities for recovery operations. From 20 to 30 minutes were required to transport the samples from the remote reactor area where the GIF is located to the Class 100 clean room facilities.

Each sample strip containing four replicate samples represents a single data point. Each of the samples from the strips was placed in a separate 50 ml beaker containing 10 ml of sterile, 0.1 percent Tween 80 water. The samples were then insonated for 2 minutes to remove the

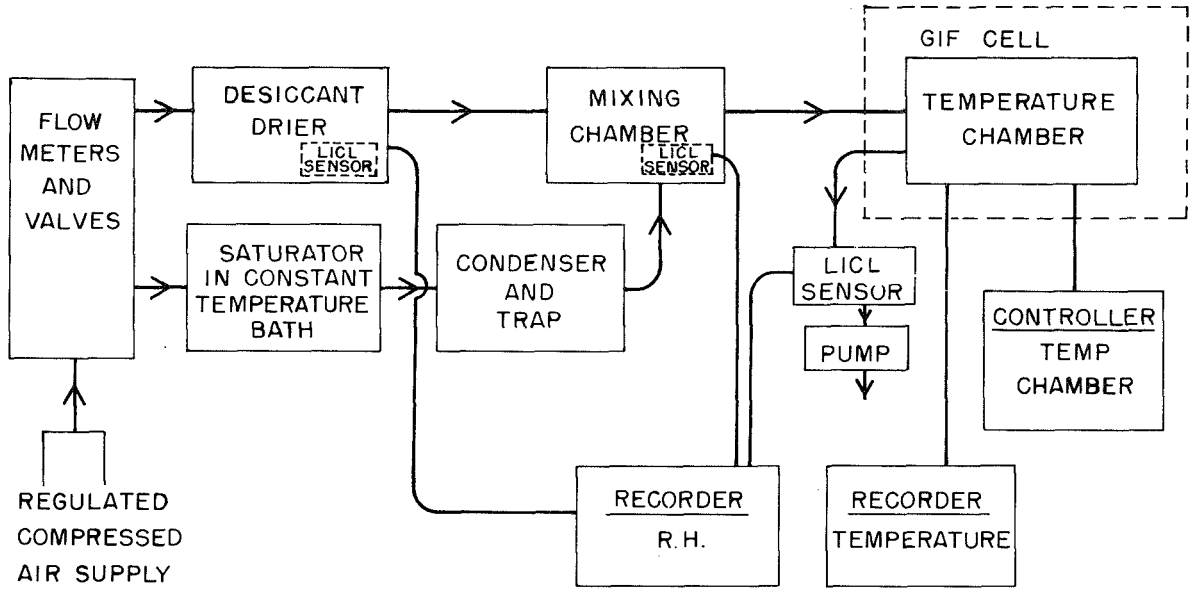

Figure 1. Equipment Set-Up for Thermoradiation Experiment with Controlled Humidity

organisms from the foil discs. Care was exercised in placing the foil discs in the beakers to assure separation of the inoculated disc and cover disc, and complete wetting and submersion of both discs in the water. During the ultrasonic treatment, occasional agitation of the beakers kept the discs separated and prevented cold welding together of the two discs. This attentive action assured good recovery of the organisms from the foil discs. The insonation is accomplished with the beakers immersed in the ultrasonic water bath to a level just above the water level in the beaker.

Additional ten-fold serial dilutions were made, as required. Dilutions were plated out in duplicate on Trypticase Soy Agar underlay, overlayed with the same type of media and then placed in an incubator maintained at 35°C. Plates were counted after 72 hours in the incubator.

Results

Thermoradiation at Various Gamma Dose Rates--A series of experiments were performed to evaluate the thermoradiation lethality under variable gamma dose rates. This data is required for optimization of heat and radiation levels to achieve effective sterilization with minimal degradation and stress.

The first experiments were performed at a temperature of 105°C. The dose rates were 2.6, 5, 13, 22, and 36 Krads/hr. The relative humidity of the air introduced into the temperature chamber was controlled at 30 ± 1 percent RH at 75°F. A comparison of the survival fraction curves of the several dose rates is shown in Figure 2. A tendency to tail is noted on the 5 and 2.6 Krads/hr curves. This behavior at low radiation dose rates will be explored further.

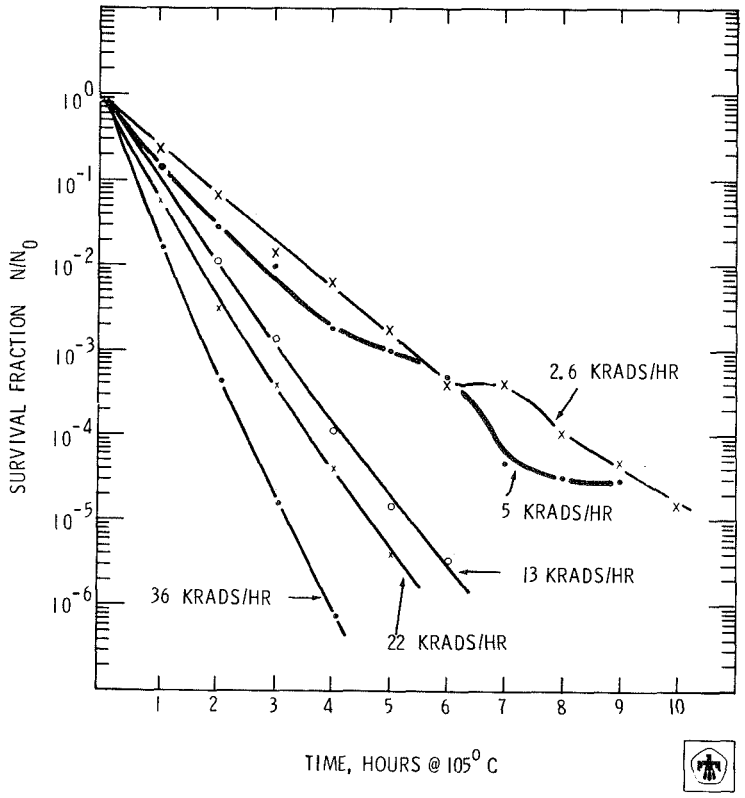

Figure 2. Thermoradiation Survival Fraction for Various Dose Rates

The interesting facet of Figure 2 is the significant difference in total radiation dose administered in each of the experiments. If we consider a four log population reduction, the total dose at the high dose rate (36 Krads/hr) was 90 Krads. At the lower dose rate (2.6 Krads/hr) the total dose to accomplish the same reduction was only 21 Krads. Thus a rather striking dependency on dose rate is indicated. The D values[2] computed from a linear regression of the variable dose rate data is plotted in Figure 3 as a function of dose rate to illustrate this dependency. In this plot of D value versus dose rate, one sees the rapid reduction in D value when small amounts of radiation are added to heat. From the ordinate, where the D value is that of dry heat alone and equal to 4.5 hours at 105°C, there is a very rapid reduction in D value as the dose rate is increased up to 10 or 12 Krads/hr. Beyond this range there is a marginal reduction in D value as the dose rate is further increased. This does permit the selection of sterilization cycles patterned around the radiation sensitivity of the item to be sterilized. For example, a somewhat optimum dose rate of about 12 Krads/hr at 105°C can be selected. This results in a D value of 1.1 hours or, based on a 12 log reduction, a sterilization cycle of 13 hours at 105°C with a total gamma dose of 156 Krads. This 13-hour cycle is a substantial reduction from the normal[3] 60 hours required to sterilize by dry heat alone at 105°C. A second option might be a higher dose rate of 36 Krads/hr. The D value in this case is 0.7 hours with a total time for a 12 log reduction of 8.4 hours and a total dose of 302 Krads. This latter option with twice the total gamma dose could in many cases be acceptable. As a matter of fact, this marginal area of higher dose rates could be valuable in the sterilization of many materials or products that are not sensitive to radiation effects. Surprisingly, it seems that most of the radiation investigations that we have found have been conducted at the higher dose rates of 100 to 1000 Krads/hr[4-11] with a few[12-13] working in the range of 20 to 100 Krads/hr and the work below 20 Krads/hr seemed directed toward the development of radioresistant cultures[16] and the inhibition of potato sprouting.[17] It does appear that the low dose rate area has been neglected. Admittedly the area of low dose rate sterilization has little significance in applications of high radiation tolerance. In spacecraft sterilization, however, thermoradiation, utilizing the most

efficient dose rate-temperature combination, could significantly improve material and component reliability. One might suspect that this same combination might be advantageous in radiopasteurization and sterilization of foods, medical products and pharmaceuticals, where loss of potency, off flavors, plastic embrittlement and carcinogenesis are presently problems.

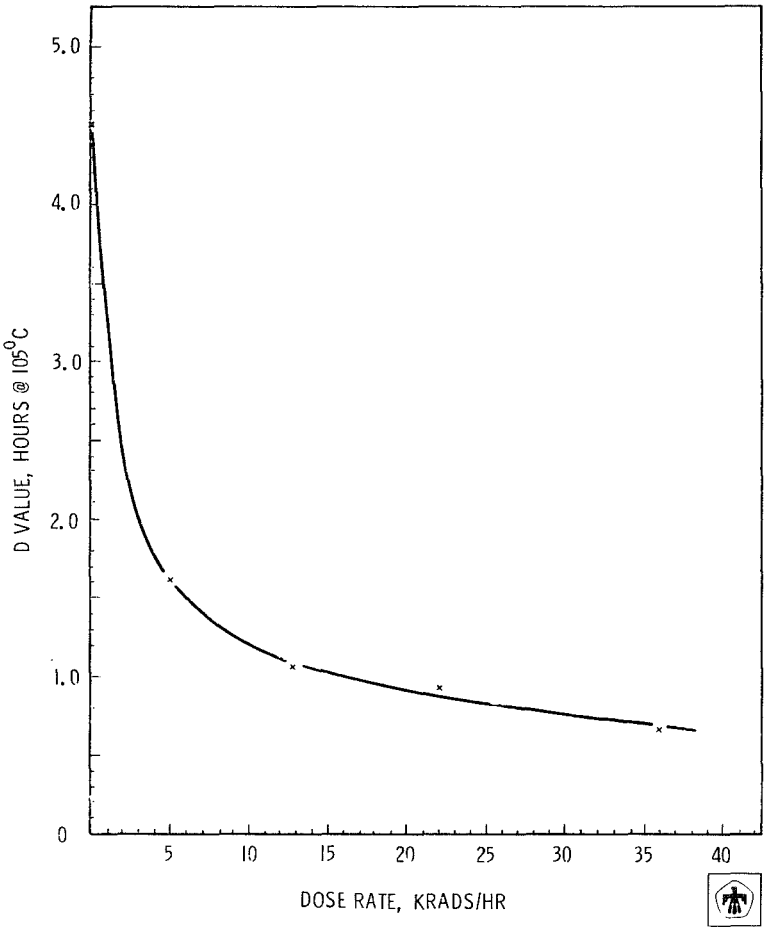

Figure 3. Thermoradiation D Value vs Gamma Dose Rate

Thermoradiation at Various Low Temperatures--A series of experiments were performed to evaluate the thermoradiation lethality at relatively low sterilization temperatures. This data combined with the variable gamma dose rate data provides further optimization of the heat and radiation levels for effective sterilization where lower temperatures are desirable.

The following illustrations (Figures 4, 5, and 6) demonstrate that good synergistic effects are available as low as 60°C. Thermoradiaton D values at a dose rate of 8.0 Krads/hr varies from 1.5 hours at 105°C[18] to 3 hours at 90°C (Figure 4) and 6 hours at 60°C (Figure 5). 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[19] depending on moisture conditions of the spores. An additional experiment was run at 95°C and 21 Krads/hr (Figure 6) with a resultant D value of 1.5 hours.

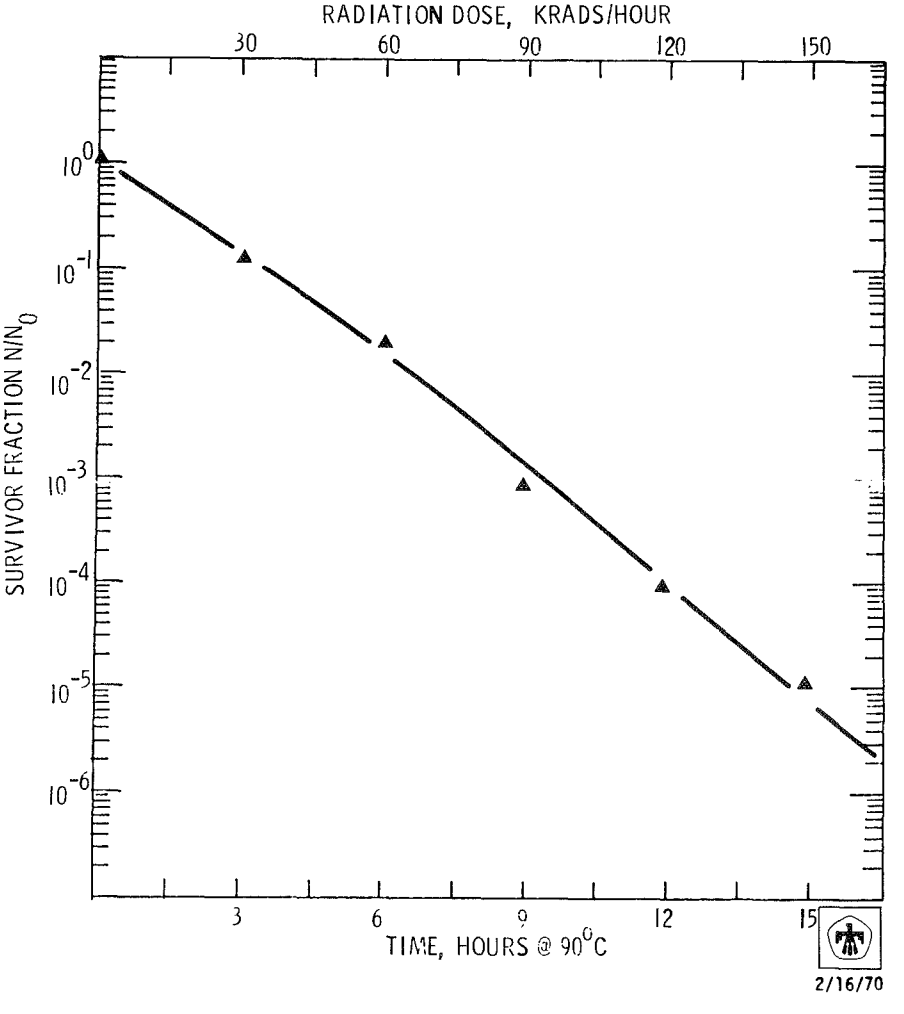

Figure 4. Thermoradiation Survivor Curve For 90°C, 30% RH and 10 Krads/hr

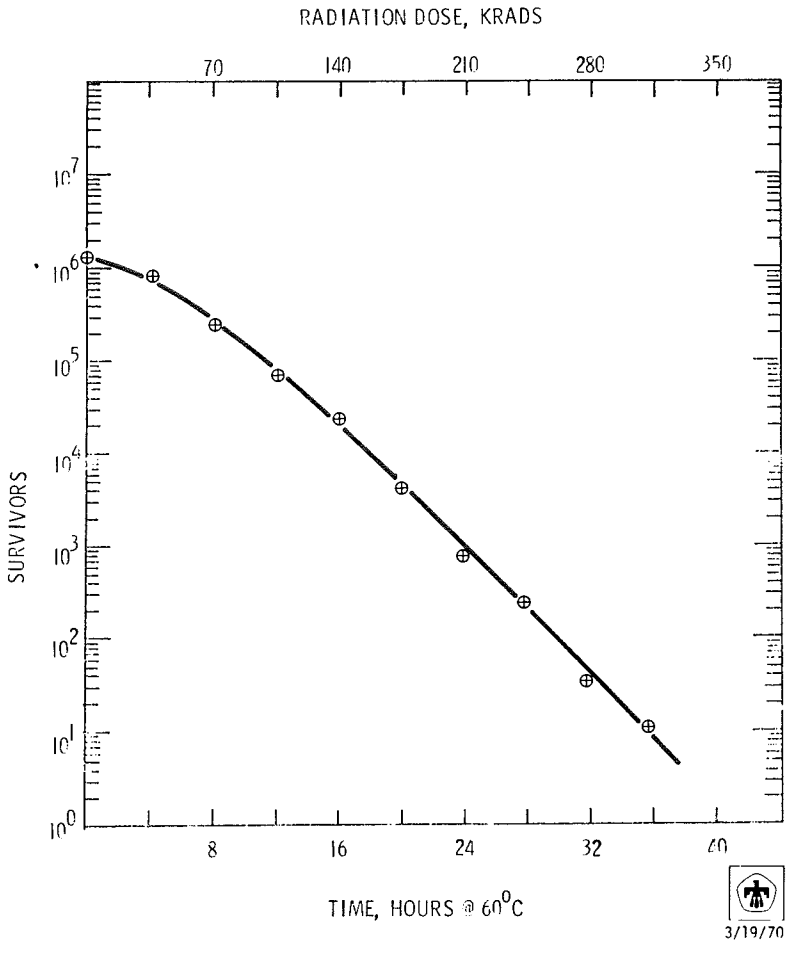

Figure 5. Thermoradiation Survivor Curve for 60°C, 30% RH (Room) and 8 Krads/hr

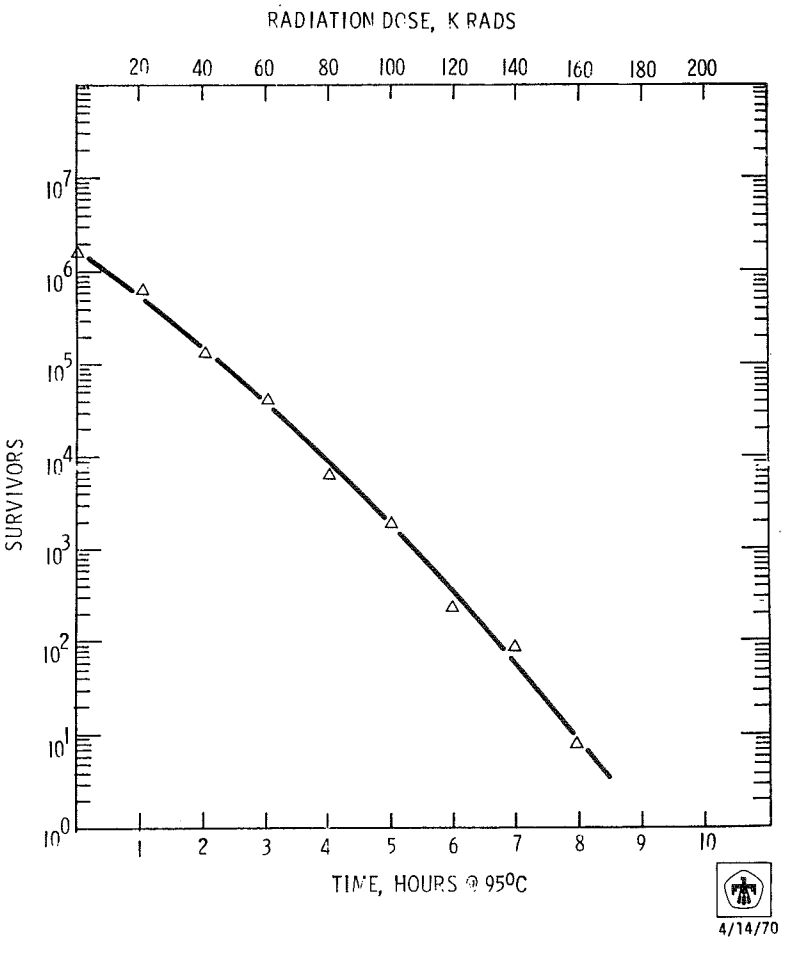

Figure 6. Thermoradiation Survivor Curve for 95°C, 30% RH (Room) and 20 Krads/hr

A valuable part of the experimentation planning is the mathematical model[20] that has been developed to predict microbial inactivation during thermoradiation exposure. All of the above D values fell closely to the model predictions. It is interesting to note that at all of these lower temperatures the D value versus dose rate curves are similar to Figure 3 with only an upward shift of the curves at lower temperatures. The knee in the curve remains at about the same dose rate value of roughly 10 Krads/hr. Thus it seems of little value to use massive dose rates if side effects of radiation are a consideration.

Using a reasonably optimum dose rate of 8 Krads/hr, Figure 7 illustrates the D value-temperature trade off that is available. In addition, it can serve to define the area of investigation. For example, if one sets an upper dose limit of 240 Krads for a 12 log population reduction, 20 Krads per log is the available dose. At 8 Krads/hr, the maximum D value would be 2-1/2 hours. The lowest practical temperatures would then be about 85° to 90°C.

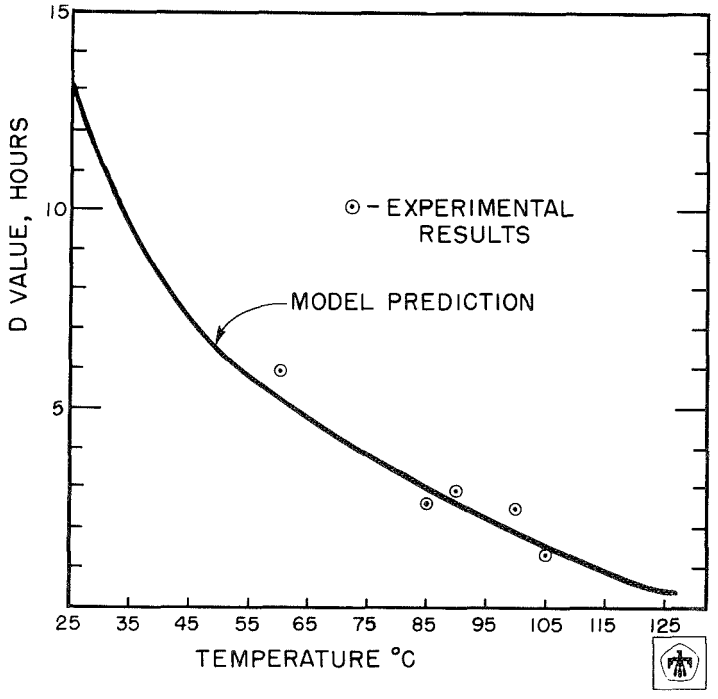

Figure 7. Effects of Temperature on D-Value at a Constant Dose Rate of 8 Krads/hr

Experimental Technique Sensitivity--The use of foil as a substrate is a somewhat recent innovation which replaces aluminum sheet planchet substrate in several applications. The need for a system that would permit considerable handling and the ability to insert samples vertically in the gamma oven facility motivated the change. A third benefit in the use of foils is the ability to increase the sample size for low levels of inoculation and survivors. Ten foils per sample are frequently used and as many as 100 per sample have been occasionally used. The foil pack however does represent a somewhat different environment than an aluminum sheet substrate that exposes the test organisms to the oven air stream. Secondly, one must consider the comparative recovery of organisms from the two configurations.

Experiments were performed to determine the effect different substrate materials would have on experimental results. The substrate materials used were aluminum foil discs and aluminum plates. Inoculation was done by electrostatic deposition[21] to insure uniformity of loading. The environments selected were 105°C, 20 Krads/hr of gamma radiation, and 60 percent relative humidity. Figure 8 shows the results comparing the two substrate materials with little significant difference noted.

In a review of 27 experiments using either foils or planchets, coefficients of variation[22] were computed for the controls only to evaluate recovery. The resultant mean coefficients were 0.176 for aluminum sheet substrate and 0.146 for the foil substrate.

A comparative experiment was also performed to determine the effects of two inoculation methods, pipeting versus electrostatic deposition. An aluminum plate substrate was used for the electrostatic deposition and aluminum foil was used for the pipetted deposition. The experimental conditions were 90°C, 10 Krads/hr and 30 percent relative humidity. The results are shown in Figure 9. The electrostatically deposited samples in all cases show a slightly higher number of survivors than the pipetted samples.

Next a check was made on the repeatability of experiments over a period of several months. A thermoradiation experiment performed in September 1969 was duplicated in February 1970 with a comparison of the data shown in Figure 10.

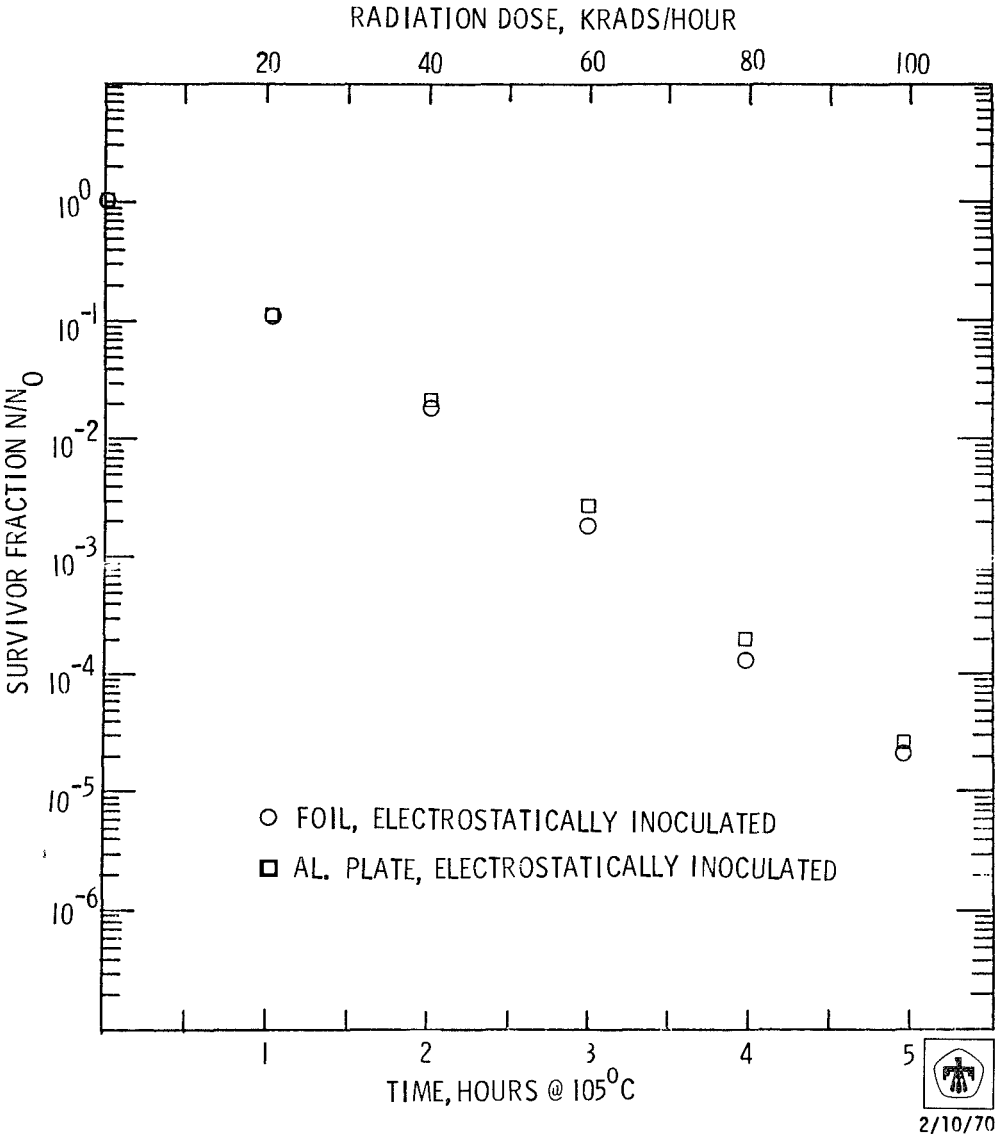

Figure 8. Thermoradiation Comparison of Foil and Aluminum Plate Substrate at 105°C, 60% RH, and 20 Krads/hr

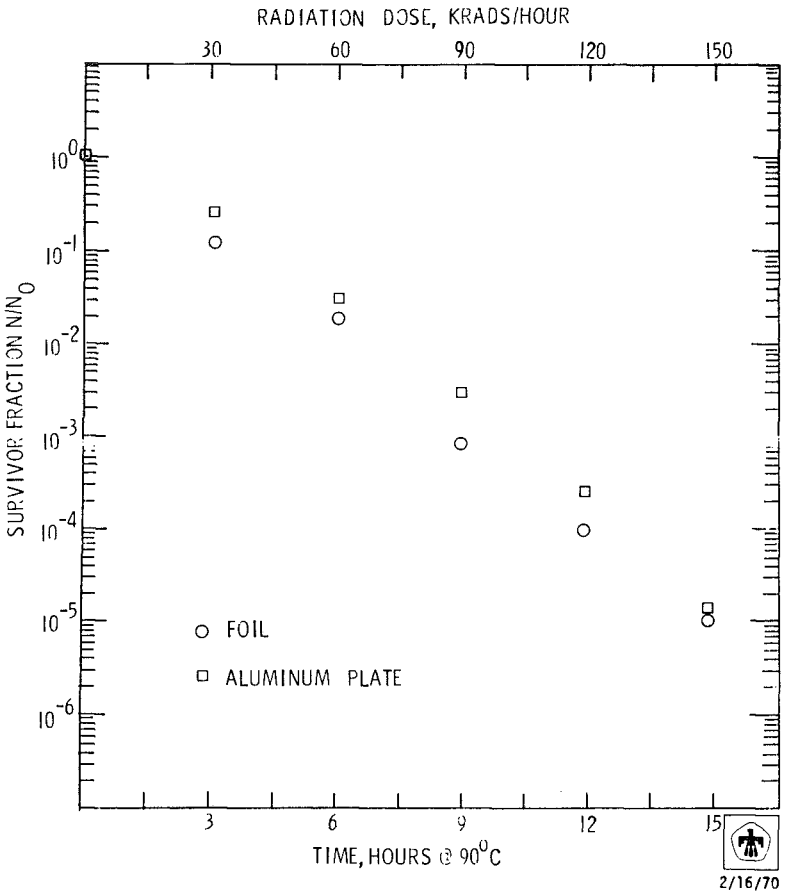

Figure 9. Thermoradiation Comparison of Foil and Aluminum Plate Substrates, Inoculation Techniques at 90°C, 30% RH and 10 Krads/hr

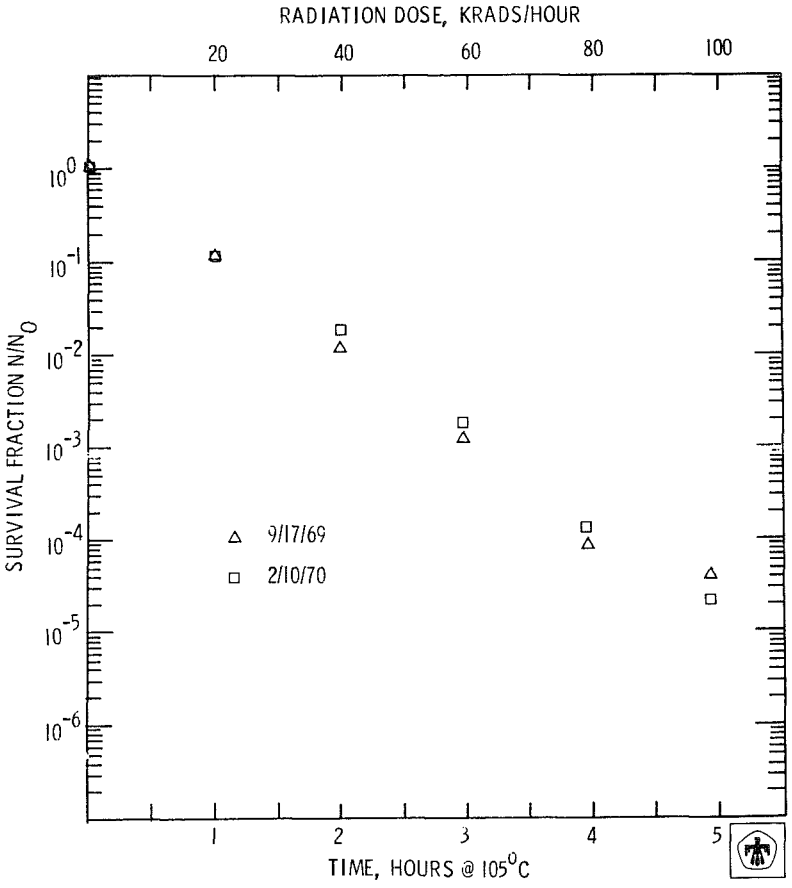

Figure 10. Repeatability of Thermoradiation at 105°C, 60% RH and 20 Krads/hr

Embedded Contamination Studies

In recent years it has been found that the heat resistance of organisms is substantially increased when they are embedded in solid materials typical of potting compounds or casting resins. In particular, Angelotti, et al[23] found the D value for B. subtilis var. niger increased to 30 hours at 105°C in methylmethacrylate. For surface contamination the D value at 105°C would be 4.5 hours. With this data as a starting point we began an evaluation of the effectiveness of thermoradiation on embedded contamination.

Materials and Methods

Preparation of Samples--The organism used was Bacillus subtilis var. niger prepared in the same manner as for surface experiments. The concentration however was 10^9 per ml in the 95 percent ethanol suspension. The spores were embedded in methylmethacrylate plastic for exposure to the sterilizing environments.

To prepare the plastic with the embedded spores, the first step was to remove the polymerization inhibitor from the liquid methylmethacrylate monomer by washing it with a 2 percent sodium hydroxide solution (NaOH). Equal volumes of the liquid monomer and NaOH solution were mixed by agitation in a separatory funnel and then allowed to separate. The NaOH solution was drawn off and the washing process was repeated, followed by two distilled water washes performed in the same manner. To remove any remaining water, the monomer was mixed with sodium sulfate (Na_2SO_4) and allowed to stand for 12 hours in a refrigerator at about 4°C.

The spore suspension was then added to the liquid monomer. Two ml of the 10^9 ethanol spore suspension were pipetted into a sterile beaker. After the ethanol had evaporated, the spores were equilibrated for 72 hours over a saturated solution of magnesium chloride ($MgCl_2$) resulting in a relative humidity of 33 percent at room temperature. Sixty ml of the prepared liquid methylmethacrylate monomer was then added to the beaker containing the spores. This mixture was insonated for two minutes at 12 watts

per square inch to break up any spore clumps in the liquid monomer. Sixty grams of methylmethacrylate powder (polymer) was added to the liquid and the mixture was placed under vacuum and stirred with a magnetic stirrer. After 18 minutes of stirring and evacuation of air from the mixture, the methacrylate was partially polymerized. This mixture was then poured out and pressed between two plate glass sheets, 12 x 8 inches to a thickness of 0.030 $\pm$ 0.001 inch. The glass sheets were sealed together and this assembly submerged in a water bath at a constant temperature of 50°C, for two hours and 45 minutes, for complete polymerization. The resulting sheets of plastic were hard, clear, free of bubbles, approximately 150 square inches in size and weighed about 100 grams.

A 5/8-inch diameter hole saw which had been ground to a very thin cutting edge was used to cut the sample chips from the plastic sheet. Average weight of these sample chips was 0.203 grams $\pm$ 0.005 and each chip contained about 1.2×10^5 viable spores. The loss of approximately one log of spores was due to the effects of polymerization. The prepared plastic chips were stored under refrigeration at 0°C or less until prepared for exposure to the sterilizing environments.

Another method[24] used to inoculate the plastic with spores was to mix the ethanol spore suspension into the dry methylmethacrylate powder. The suspension was poured over 30 grams of methylmethacrylate powder and mixed for 5 minutes on a magnetic stirrer. The mixture was allowed to air dry at room temperature until all the ethanol had evaporated and then stirred again until the spore inoculum was well distributed throughout the powder. The inoculated powder was then equilibrated over a saturated solution of $MgCl_2$ at a relative humidity of 33 percent at room temperature for 72 hours. Thirty ml of the prepared methylmethacrylate liquid monomer was added to the powder mix and this mixture placed under vacuum and stirred with a magnetic stirrer. When air bubbles no longer emerged from the mixture, it was removed from the vacuum and heated for 30 seconds at 50°C, to initiate polymerization. The mixture was then returned to the vacuum chamber and evacuated and stirred until it was partially polymerized. The plastic was

then cast between the plastic glass sheets, polymerized and the sample chips cut in the same manner as described above.

To assemble the samples for exposure, three of the chips were placed on an aluminum strip and secured in place with a narrow strip of autoclave tape. The assembled sample strips were maintained under refrigeration at less than $0^{o}C$ until exposed to the sterilizing environments.

Exposure Methods--The thermal and irradiation environments were provided by the same facilities described under surface contamination studies. The relative humidity was the ambient condition in the GIF cell where the temperature chamber was located. This RH varied from 20 percent to 30 percent during the period of the experiments. Silver phosphate or cobalt glass dosimeters, depending on the dose, were placed on selected sample strips to verify the computed dose rate.

Recovery Methods--Each sample strip, when removed from the temperature chamber after the desired exposure, was wrapped in sterile aluminum foil and returned to the clean room facilities for recovery operations.

Each sample strip with the three chips represents a single data point. The sample chips were removed from the metal strip and each placed in a perforated metal basket which was in a wide mouth dilution bottle containing 100 ml of sterile acetone. The bottles were capped and placed horizontally on a shaker table. The metal basket kept the plastic chip suspended in the acetone during the agitation process on the shaker which facilitates dissolving the plastic chip. The dissolution operation required approximately 2 hours depending on the time the chips were exposed to the irradiation environment. After dissolution, tenfold serial dilutions of the solution were prepared as required with sterile acetone. The dilutions were pipetted onto sterile filters in a filter holder apparatus and vacuum filtered. The filters used were Gelman Metricel, alpha 6, with a pore size of 0.45 micron. The filtered spores were first washed with sterile acetone to rinse off any methylmethacrylate residue, followed by two sterile water rinses to remove the acetone. The filter containing the spores was then

placed on a Trypticase Soy Agar underlay in a petri dish and placed in the incubator. The plates were counted after 24 hours of incubation at 35°C.

Results

The two methods of preparation previously described yielded differing results. First of all, the latter method of inoculating the dry polymer resulted in rather large clumps of spores in the final sample material. These clumps seemed to contain on the order of 10^3 spores each. The survivor curve, using this method, is shown in Figure 11 and is labeled clumped spores. A linear regression of the data shown results in a D value of 2.5 hours. If we, however, only consider that part of the curve with the minimum slope the D value would be about 6-1/2 hours. A second experiment using methacrylate with spores uniformly distributed in the material but with the same temperature/dose rate conditions yielded an overall D value of 1.9 hours. Considering only the latter part of the curve the D value was 4 hours. The reason for the initial slope has not been determined. It may be due in part to additional polymerization but a similar response was obtained using gelatine matrix equilibrated at 75 percent RH before exposure to thermoradiation treatment. Figure 12 is a comparison of two dose rates with spores uniformly distributed in the methacrylate. These two curves are the first in defining a D value versus dose relationship at 105°C for methacrylate. An increase of 150 percent in the dose rate reduced the overall D value by 25 percent.

Conclusion

A means for selecting the most appropriate balance between temperature and gamma radiation has been demonstrated. The striking dose rate dependency of radiation sterilization suggests the greatest potential in low dose rate sterilization to minimize side effects. The longer exposure times associated with low dose rates fortunately can be reduced by the addition of heat. Thus thermoradiation at low temperatures and low levels of gamma radiation is indeed a means for sterilization whereby the degradation to spacecraft, components, materials and products can be minimized.

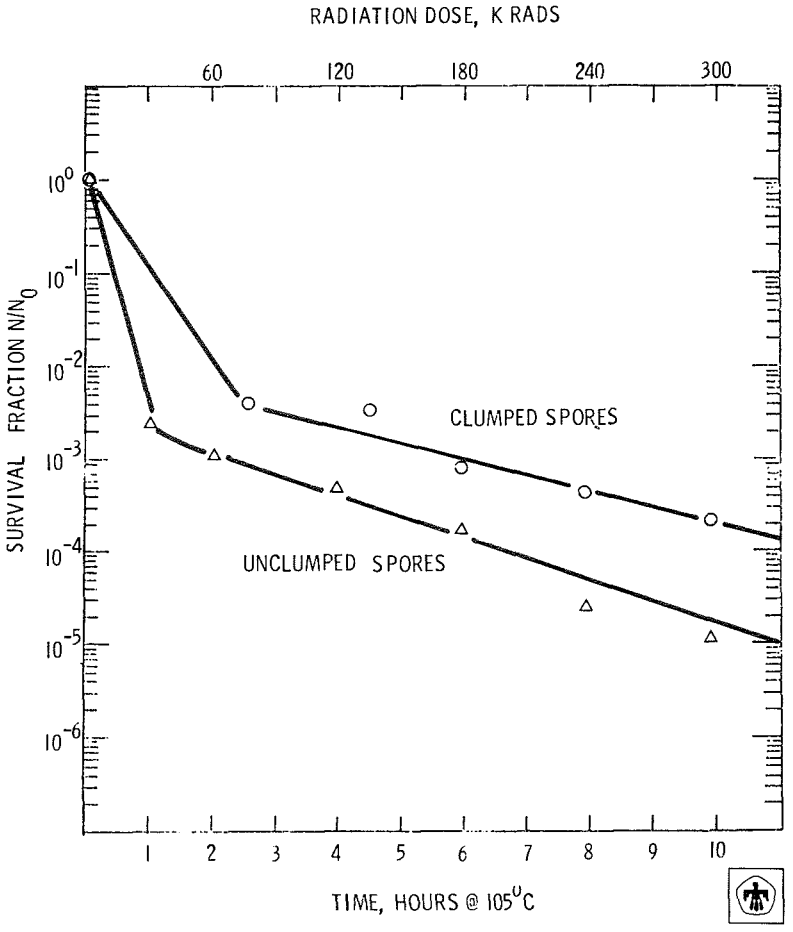

Figure 11. Thermoradiation Survivor Curve for 105°C B. subtilis in methacrylate at 30 Krads/hr

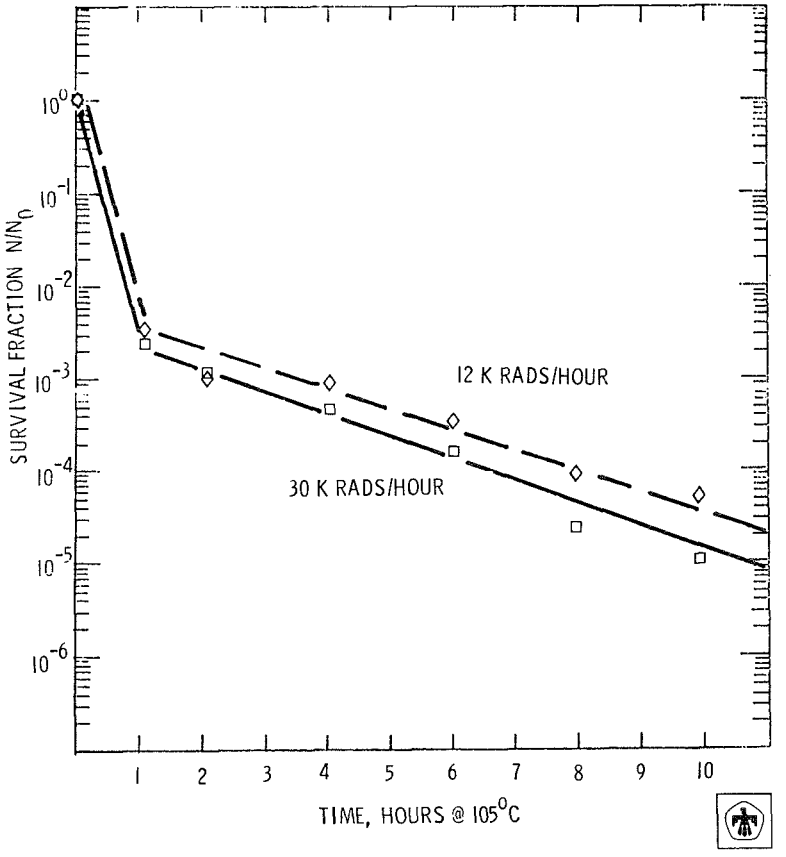

Figure 12. Thermoradiation Survivor Curve for 105°C B. subtilis in Methacrylate

NOTES AND REFERENCES

1. Garst D. M., Lindell, K. R., The Development of Two Closely Controlled Humidity Systems, SC-RR-70-409, Sandia Laboratories, Albuquerque, New Mexico, June 1970.

2. A "D" value is the time required for a given microbial population to be reduced by 90 percent or one log in population at a given temperature. The D value can also be the radiation dose required to reduce a given population by one log.

3. Spacecraft Sterilization Technology, NASA SP-108, 1966.

4. Graikoski, John, The Simultaneous Lethal Effect of Temperature and Gamma Radiation on Bacterial Spores, Univ. Microfilms Ltd., Ann Arbor, Michigan, p. 35, 1961.

5. Tallentire, A., and Powers, E. L., "Modification of Sensitivity to X-irradiation by Water in Bacillus megaterium, Rad. Res. 20 (1963), pp. 270-287.

6. Briggs, A., "The Resistance of Spores of the Genus Bacillus to Phenol, Heat, and Radiation," J. Appl. Bact. 29(3): 490-504, 1966.

7. Roberts, T. A., "Heat and Radiation Resistance and Activation of Spores of Clostridium welchii," J. Appl. Bact. 31 (1968) pp. 133-144.

8. Ingram, M., and Thornley, M. J., "The Effect of Low Temperature on the Inactivation by Ionizing Radiations of Clostridium botulinum Spores in Meat," J. Appl. Bact. 24(1): 94-103, 1961.

9. Licciardello, J. J., and Nickerson, J. T. R., "Effect of Radiation Environment on the Thermal Resistance of Irradiated Spores of Bacillus subtilis," Appl. Microbiol. 11 (1963), pp. 216-219.

10. Anellis, A., Cichon, C. J., and Rayman, M. M., "Resistance of Bacillus coagulans Spores to Gamma Rays. Application of the Multiple Tube Probability Method," Food Res. 25(2): 285-295, 1960.

11. Licciardello, J. J., and Nickerson, J. T. R., "Effect of Radiation Environment on the Thermal Resistance of Irradiated Spores of Clostridium sporogenes," J. Food Sci. 27(3): 211-218, 1962.

12. Midura, T. F., Graikoski, J. T., Kempe, L. L., and Milone, N. A., "Resistance of Clostridium perfringers, Type A Spores to Ionizing Radiation," AIBS Bull. 13(4): 53, 1963.

13. Koesterer, Martin G., "Thermal Death Studies on Microbial Spores and Some Considerations for the Sterilization of Spacecraft Components," Dev. Indust. Microbiol. 6 (1964), pp. 268-275.

14. Hill, E. C., and Phillips, G. O., "The Inactivation of Bacillus subtilis Spores in Penicillin by Gamma Radiation," J. Appl. Bact. 22(1): 8-13, 1959.

15. Powers, E. L., Webb, R. B., and Ehret, C. F., "Storage, Transfer, and Utilization of Energy from X-rays in Dry Bacterial Spores," Rad. Res. Supplement 2 (1960), pp. 94-121.

16. Licciardello, J. J., Nickerson, J. T. R., and Goldblith, S. A., Effect of Repeated Treatment with Gamma Rays on the Radioresistance, Virulence, and Culture Characteristics of Certain Pathogenic Bacteria, Mass. Institue of Technology, Cambridge, Massachusetts, October 1968

17. Errington, R. F. and MacQueen, K. F., Gamma Irradiation of Potatoes to Inhibit Sprouting, Canadian Potato Industry Fifth Annual Conference, University of Brit. Col., Commercial Products, Atomic Energy of Canada Ltd., Ottawa, Canada, 1961.

18. Reynolds, M. C., The Feasibility of Thermoradiation for Sterilization of Spacecraft--A Preliminary Report, SC-RR-69-857, Sandia Laboratories, , Albuquerque, New Mexico, December 1969.

19. Semiannual Report, School of Public Health, University of Minnesota, December 1969

20. Dugan, V. L., A Mathematical Model for Thermoradiation Inactivation of Dry Bacillus subtilis var. niger Spores, SC-RR-70-203, Sandia Laboratories, Albuquerque, New Mexico, April 1970.

21. Whitfield, W. J., Electrostatic Deposition Device to Deposit Mono-Layers of Bacterial Spores on Test Surfaces, SC-R-70-4259, Sandia Laboratories, Albuquerque, New Mexico, April 1970.

22. Coefficient of variation is defined as the ratio of standard deviation to the mean.

23. Angelotti, R., et al, Appl. Microbiol. 16(5): 741, May 1968.

24. Ibid.

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