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Effect of Microwaves on *Escherichia coli* and *Bacillus subtilis*

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Suspensions of *Escherichia coli* and *Bacillus subtilis* spores were exposed to conventional thermal and microwave energy at 2,450 MHz. The degrees of inactivation by the different energy sources were compared quantitatively. During the transient heating period by microwave energy, approximately a 6 log cycle reduction in viability was encountered for *E. coli*. This reduction was nearly identical to what is expected for the same time-temperature exposure to conventional heating. Heating of *B. subtilis* spores by conventional and microwave energy was also carried out at 100 C, in ice and for transient heating. The degree of inactivation by microwave energy was again identical to that by conventional heating. In conclusion, inactivation of *E. coli* and *B. subtilis* by exposure to microwaves is solely due to the thermal energy, and there is no per se effect of microwaves.

There have been several publications in recent years which have mentioned some possible microbicidal effects of microwaves other than those solely due to heat (3–5). An analysis of these papers indicates that in every case there is a lack of specific quantitative data to verify these conclusions. On the other hand, there are papers such as that of Brown and Morrison (2) which state "no significant destruction of bacteria in aqueous solution occurs from application of radio-frequency fields in the frequency range up to 600 megacycles, except of course the destruction brought about by thermal effects."

The work described herein was initiated to ascertain whether microwaves at a frequency of 2,450 MHz have a specific bactericidal effect other than that due to heat. This work utilizes the quantitative methods of measuring microbial survival and of studying the effects of conventional thermal energy on the organisms. Two species were studied, *Escherichia coli* and *Bacillus subtilis* spores, and the data are reported here.

MATERIALS AND METHODS

Time-temperature profile of solutions heated in the radar oven. The radar oven used throughout the experiments was Radarange, model no. 1161, series no. 6 (2,450 MHz; Raytheon Co., Hooksett, N.H.). Samples to be heated in the oven were placed in 150-ml Pyrex beakers. These beakers were approximately 5.6 cm in diameter and 8 cm in height. To obtain the time-temperature profiles during heating, 9.1 ml of solution in a 150-ml beaker was placed in the cavity of the radar oven. The beaker was placed in the geometric center of the radar oven and was supported by a metal pan, approximately 11.5 cm high, 50 cm long, and 43 cm wide. After heating for a designated length of time, the solution temperature was measured by use of a 0 to 110 C thermometer marked in 0.1 C intervals. Experiments were also performed to account for the decrease in temperature of the solution due to heat losses after removing the solution from the Radarange.

Inactivation of E. coli. E. coli strain B6 was used for all experiments. The organisms were cultivated in 500-ml Erlenmeyer flasks containing 150 ml of Nutrient Broth (Difco). The cultures were incubated at 37 C on a rotary shaker for 18 hr. The suspension was then cooled to 4 C and was centrifuged for 40 min at 2,500 rev/min in an International model 2 centrifuge. The supernatant fluid was discarded and the packed cells were washed with 150 ml of cold (4 C) 0.067 M phosphate buffer (*p*H 7). The suspension was then recentrifuged for 40 min at 2,500 rev/min. The supernatant fluid was again discarded, and the cells were resuspended in 150 ml of 4 C phosphate buffer. The cell suspension was kept refrigerated until use.

To determine the thermal inactivation of *E. coli* by heat, the Stern and Proctor (6) apparatus was employed. Exactly 0.025 ml of the bacterial suspension was placed in 150-mm melting-point capillary tubing. The sealed tube was then placed in a constant temperature bath for appropriate lengths of time. After heating, the tube was cooled immediately in an ice bath. The degree of inactivation was determined by plate counts on nutrient agar incubated at 37 C for 24 hr.

The following procedure was used to determine the degree of inactivation of *E. coli* by microwaves. A

9.1-ml amount of the bacterial suspension was placed in a 150-ml beaker which was kept in the microwave oven for the designated time, then 1.0 ml of the suspension was removed and sprayed into 9 ml of refrigerated phosphate buffer. The degree of inactivation was determined by viable plate counts as stated above.

Inactivation of B. subtilis var. niger. The following procedure was used to prepare spores of *B. subtilis* var. *niger*. The sporulation medium was composed of: yeast extract, 0.5%; Casamino Acids, 0.1%; glucose, 0.25%; $MnSO_4$, 10 ppm; $FeSO_4$, 0.001%; agar, 3%; at *p*H 6.8. Sporulation was complete after incubation for 5 days at 30 C. At this time, microscopic examination showed the population to be approximately 90 to 95% spores. The spores were harvested from agar plates by gentle scraping with glass rods after flooding each plate with 10 ml of cold sterile distilled water. The suspension was centrifuged in a refrigerated Sorvall RC2-B centrifuge for 15 min at 15,000 rev/min, followed by a second washing and centrifugation. The harvested spores were stored in 0.067 M phosphate buffer at 4 C until used.

In view of the higher thermal resistance of *B. subtilis* spores than that of *E. coli*, the capillary tubing technique of determining thermal death time of this organism was not employed. The first method, outlined below, was used to compare the degree of inactivation during the transient heating period. From previous experiments, the time-temperature profile of the phosphate buffer solution during residence in the Radarange was known. Therefore an attempt was made to approximate the same time-temperature profile by heating the spore suspension over an open flame. Several attempts were made, adjusting the intensity of the flame so that the time-temperature profile obtained in the Radarange was approximated. During heating over the open flame, 1 ml of the suspension was removed at various times and cooled instantly in 9 ml of refrigerated buffer. Viable counts were determined by use of tryptone-glucose-extract agar, and incubation was at 30 C for 24 hr.

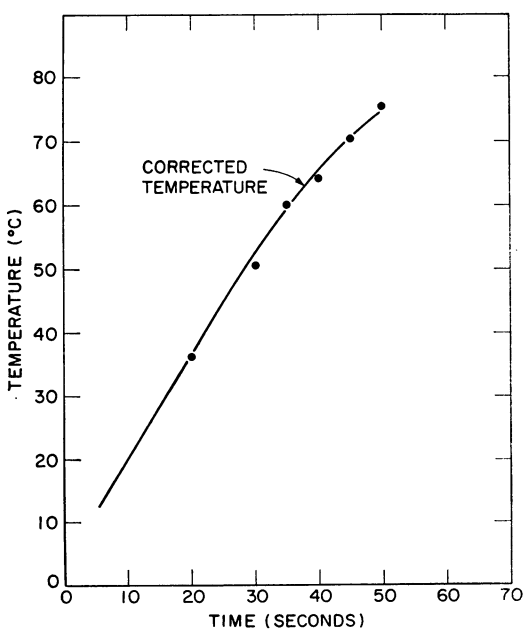

FIG. 1. *Time-temperature heating profile of Escherichia coli in 0.067 M phosphate buffer by microwave energy.*

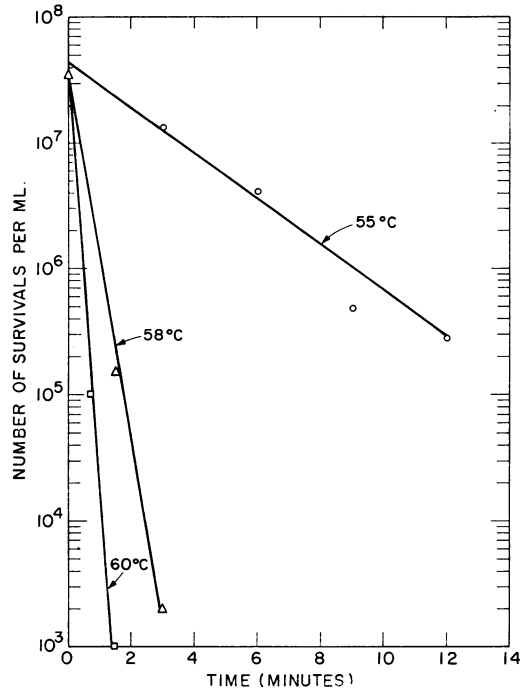

FIG. 2. *Thermal inactivation of Escherichia coli in 0.067 M phosphate buffer.*

The second method used for comparison on the degree of inactivation of *B. subtilis* by heat and radar waves was performed at the boiling temperature. Appropriate volumes of *B. subtilis* suspension were heated to boiling in the Radarange and over an open flame. At different time intervals, 1 ml of the heated suspension was removed and was placed instantly into 9 ml of refrigerated buffer. The viability of the heated samples was enumerated by use of the technique outlined above.

RESULTS AND DISCUSSION

Time-temperature profile for radar heating. The corrected time-temperature profile of 0.067 M phosphate buffer containing *E. coli* is shown as Fig. 1. This profile will be used below to calculate the inactivation of *E. coli* due solely to heat in the Radarange.

Inactivation of E. coli B6. The thermal inactivation of *E. coli* B6 in 0.067 M phosphate buffer at various temperatures is shown in Fig. 2. In view of the extreme sensitivity of *E. coli* to heat, it was not

possible to obtain accurate inactivation studies at temperatures greater than 60 C. The data obtained from the capillary tubing inactivation studies were plotted according to the Arrhenius equation.

$$k = Ae^{-\Delta E/RT} \quad (1)$$

where k = inactivation rate constant, minutes^{-1}; A = collision frequency factor, minutes^{-1}; ΔE = activation energy, calories per gram-mole; R = gas constant, calories per gram-mole °K; T = absolute temperature, °K. This plot is shown as Fig. 3. Also shown on this plot are the data of Aiba, Humphrey, and Millis (1) for the inactivation of *E. coli* in the same phosphate solution. Excellent agreement between the two studies was obtained.

Inactivation of E. coli by heat and radar waves. The inactivation of *E. coli* exposed for various times in the Radarange is shown in Fig. 4. On the same graph, the theoretical inactivation values due to heat alone are also shown. The latter values were calculated in the following manner.

The rate of inactivation of *E. coli* due to heat obeys first-order kinetics according to the following equation.

$$dN/dt = -kN \quad (2)$$

where N = concentration (number per milliliter)

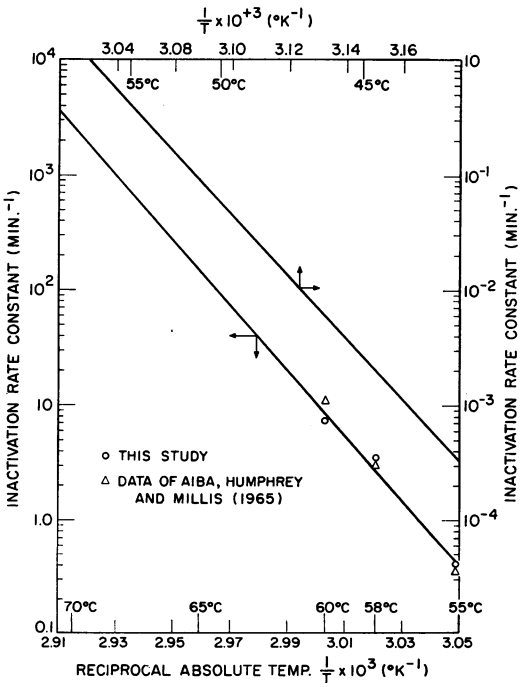

FIG. 3. *Temperature dependence of the inactivation rate constant of Escherichia coli in 0.067 M phosphate buffer.*

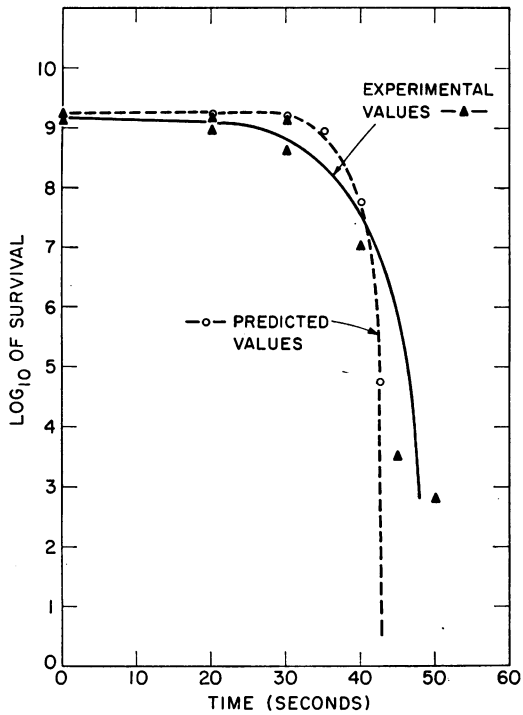

FIG. 4. *Inactivation of Escherichia coli in 0.067 M phosphate buffer in Radarange.*

of viable organisms at any time t; t = time (seconds); k = inactivation rate constant (seconds^{-1}).

The dependence of the inactivation rate constant on temperature can be expressed by the Arrhenius Equation (*see* Fig. 3 and equation 1). By separation of the variables of equation 2, the degree of inactivation can be evaluated from the following definite integral.

$$-\int_{N_0}^{N} \frac{dN}{N} = \int_0^t k\,dt \quad (3)$$

By use of the time-temperature heating profile of Fig. 2 in conjunction with the Arrhenius plot of Fig. 4, the value of k can be obtained for any time t. It is therefore possible to predict the degree of inactivation during transient heating by graphically integrating the right side of equation 3.

The survival data obtained in the heating of phosphate buffer suspension of *E. coli* in the Radarange (represented by the triangles in Fig. 4) were nearly identical to the expected survival curve based on the data obtained in the conventional heating studies (represented by the circles in Fig. 4). Therefore, the inactivation of *E. coli* when exposed to radar waves appears to be caused solely by the heating effect.

TABLE 1. *Inactivation of Escherichia coli in the Radarange with ice added to the bacterial suspension*

Time in Radarange (sec)	Vol of *E. coli* suspension (ml)	Wt of ice added (g)	Final temp of suspension (C)	Bacteria count (no./ml)	
				Before exposure	After exposure
50	9.1	20	20	3.9×10^8	4.1×10^8
70	9.1	35	29	2.6×10^8	2.0×10^8
100	9.1	40	51.5	2.2×10^8	2.5×10^8

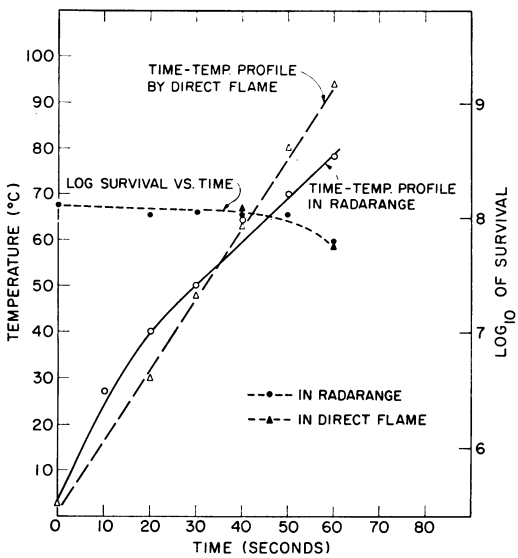

FIG. 5. *Inactivation of Bacillus subtilis spores in 0.067 M phosphate buffer in Radarange and in direct flame.*

To substantiate these data further, an additional set of experiments was performed with different amounts of ice added to the *E. coli* suspension prior to heating in the Radarange. The ice was added to the bacterial suspension to obtain a longer exposure time in the radar field while maintaining a lower overall temperature. The mixture of ice and buffer was heated in the Radarange for various periods of time. The results of these studies are in Table 1.

It appears that exposing *E. coli* to radar waves up to 100 sec did little or no damage in terms of the viability. These studies reinforce the previous conclusion that the inactivation of *E. coli* by 2,450 MHz microwaves is due solely to the thermal effect.

Inactivation of B. subtilis spores by heat and radar waves. Inactivation studies with *B. subtilis* spore suspensions were conducted during transient heating in the Radarange as well as during direct heating with an open flame. As previously stated, the time-temperature profile during direct heating was approximately the same shape as that obtained in the Radarange. The results are plotted in Fig. 5. During the transient heating period, the degree of inactivation due to radar waves and direct heating was identical. It therefore appears that the small amount of inactivation occurring in 60 sec is caused solely by the thermal effect.

A second set of experiments was also performed wherein the *B. subtilis* suspensions were allowed to boil in the Radarange as well as by direct heating with an open flame. The results are plotted in Fig. 6. The trend of the results again indicates that radar waves per se have little or no influence on the inactivation of *B. subtilis* spores. However, these studies may be inaccurate, owing to evaporation of the solutions during heating.

Energy input by microwaves. The rates of energy absorption by the different solutions used can be calculated from the time-temperature heating profile of Fig. 1. By use of the linear portions of the profiles, the maximal energy absorption rates for these solutions in the microwave field were tabulated (Table 2). This table was constructed to provide an estimate of the energy

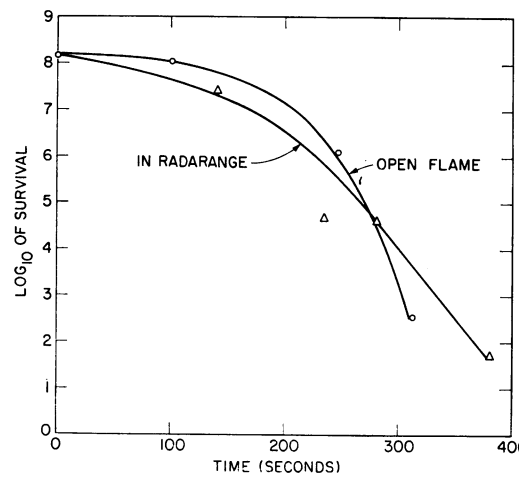

FIG. 6. *Inactivation of Bacillus subtilis in 0.067 M phosphate buffer in Radarange and over open flame.*

TABLE 2. *Energy absorption rates in microwave oven*

Type of solution	Energy absorption rate	
	Cal/sec	Watts/9.1 ml
0.1 M KH_2PO_4	14.7	3.52
E. coli and *B. subtilis* in 0.067 M KH_2PO_4 and Na_2HPO_4 buffer	15.4	3.79

absorption rates during microwave heating of the different solutions used in these experiments.

The basic conclusion of this work is that microwaves at 2,450 MHz per se cause no destruction of *E. coli* when held below 51 C. The inactivation occurring when *E. coli* is exposed to 2,450 MHz microwaves is due to heat. This conclusion can also be drawn from the work on *B. subtilis* spores.

ACKNOWLEDGMENT

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