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Mechanism of Microwave Sterilization in the Dry State

DAVID K. H. JENG,* KURT A. KACZMAREK,† ARCHIE G. WOODWORTH, AND GLENN BALASKY

Advanced Sterilization Technology, Baxter Travenol Laboratories, Inc., Round Lake, Illinois 60073

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With an automated computerized temperature control and a specialized temperature measurement system, dry spores of *Bacillus subtilis* subsp. *niger* were treated with heat simultaneously in a convection dry-heat oven and a microwave oven. The temperature of the microwave oven was monitored such that the temperature profiles of the spore samples in both heat sources were nearly identical. Under these experimental conditions, we unequivocally demonstrated that the mechanism of sporicidal action of the microwaves was caused solely by thermal effects. Nonthermal effects were not significant in a dry microwave sterilization process. Both heating systems showed that a dwelling time of more than 45 min was required to sterilize 10^5 inoculated spores in dry glass vials at 137°C. The *D* values of both heating systems were 88, 14, and 7 min at 117, 130, and 137°C, respectively. The *Z* value was estimated to be 18°C.

Electromagnetic energy in the microwave region (225 MHz to 100 GHz, typically 2,450 MHz) is extensively studied as one of the alternative energy sources for sterilization. The efficiency of microwave sterilization is essentially a function of both the electromagnetic field strength and the exposure time. The electromagnetic energy is expressed largely in two forms: (i) the factors that depend on the dielectric properties of the dipole molecules of the irradiated materials in the form of heat (thermal effect) and (ii) the factors that do not depend on the dipole molecules in the form of a direct effect of the radiofrequency (nonthermal effect).

Although studies to differentiate the thermal and nonthermal effects of microwaves on microbiological systems have been attempted, the mechanism of sporicidal action by microwaves has not been resolved. A few recent studies have conflicting conclusions. Olsen et al. believe that the nonthermal effect of microwaves plays a role in inactivation of microorganisms in suspension via formation of hydrogen peroxide and other chemical transformation of small molecules such as chemical bond cleavage (15). However, details of the evidence put forward to support these conclusions have not been given. Culkin and Fung observed that heat generated during the microwave exposure alone is inadequate to fully account for the nature of the lethal effects of microwaves for microorganisms in soup (5). Goldblith and Wang (8) and Lechowich et al. (10), however, found that in liquid systems the bactericidal and sporicidal activities caused by microwaves were caused solely by thermal effects. Recently, Furia et al. found essentially similar results with yeast cells (7).

All biological systems are electrochemical in nature. It would, therefore, not be surprising if electromagnetic fields influenced the physiology of microorganisms. However, a liquid suspension containing microorganisms is not an efficient system for the purpose of studies because of its reduction of the local electric field strength (21). In addition, a microbiological liquid sample placed in an electromagnetic field rises in temperature as dielectric absorption converts electrical energy into heat, which could mask the nonthermal functions. Therefore, in any experimental attempt to differentiate the thermal and nonthermal effects of microwaves on microbial physiology, the microbiological samples should be in a dry state. It is critically important that the microwave-heated and non-microwave-heated samples are simultaneously studied under the same temperature profiles.

Vela and Wu (22) studied the mechanism of microwave bactericidal action under dry conditions at a subbactericidal temperature and concluded that the dry or lyophilized microorganisms are not capable of absorbing microwave energy and are not damageable by the microwaves. Wayland et al., however, used microwaves to heat the dry spores in comparison with convection thermal heating and found that the thermal and electromagnetic nonthermal functions of microwaves are interdependent (23).

Several investigators have proposed the use of microwaves for sterilizing materials in the dry state for medical use (12, 18, 19) (U.S. patent 3,753,651; Japanese patent 47860). Particularly intriguing is the possibility of sterilizing heat-sensitive products at relatively low temperatures for a short time. Lohman and Manique recently reported that a microwave treatment for as short as 2.7 min in a constantly rising temperature up to 140°C is effective to sterilize 10^6 *Bacillus subtilis* subsp. *niger* spores in well-insulated and properly loaded dry glass vials (12). Similar results are claimed in U.S. and Japanese patents. Generally, a typical dry-heat sterilization process by convection heat would require a few hours of dwelling time at 160°C.

The effect of heat sterilization is a function of temperature and exposure time. If microwaves can sterilize dry products more efficiently in a shorter time and at lower temperatures than convection heat under the same humidity conditions, the effect of the sporicidal activities of microwaves must be caused by the nonthermal effects, rather than or in addition to the thermal effects. The quantal energy of microwaves per se (about 10^{-5} eV) is not sufficient to cause chemical bond cleavage (11, 20). Effects of microwaves on biological systems other than chemical bond cleavage have been reported (2–4, 25) and reviewed (L. Furia, Ph.D. dissertation, University of Utah, Salt Lake City, 1986). If the nonthermal effects of microwaves can cause bactericidal and sporicidal

* Corresponding author.
† Present address: Department of Electrical and Computer Engineering, University of Wisconsin, Madison, WI 53706.

activities, they must be a result of nonthermal effects other than chemical bond cleavage.

In conducting mechanism studies of microwaves, perfect temperature control is of prime importance since even a temporary temperature variation of only a few degrees may completely obscure the results. By using a computerized temperature control and a specialized temperature measurement system, experimental conditions were created in the current study so that dry spores were simultaneously exposed to microwaves and to convection dry heat with nearly identical temperature profiles. Under these conditions, the possibility of developing a short sterilization cycle at a lower temperature by microwaves to inactivate the dry spores was evaluated and the mechanism of the sporicidal activities of microwaves was studied.

MATERIALS AND METHODS

Bacterial spores. The spores of *B. subtilis* subsp. *niger* (ATCC 9372) were used because of their extreme resistance to dry heat (9). The dry-heat resistance is related to water activity in spores. The optimal water activity for the maximal heat resistance is about 20 to 40% relative humidity (1). A 10^7/ml spore suspension (10 μl) in distilled water was inoculated on the inside side wall of borosilicate glass vials (65 by 15 mm diameter) approximately 1 cm from the bottom and air dried. The vials were then situated in a closed chamber containing a beaker of saturated calcium chloride, which provided 33% relative humidity at 22°C (24), for 7 days before being used. The relative humidity was verified by a calibrated humidity sensor (model HM 31; Vaisala, Inc.).

Spore recovery. After heat exposures, 5 ml of sterile distilled water was added to the sample vials, which were then sonicated for 15 min in an ultrasonic bath. Duplicates of 1-ml samples were assayed by the standard pour plate methods in Trypticase soy agar (BBL Microbiology Systems). All plates were incubated at 30 to 35°C for 48 h.

Convection oven. An electric dry-heat convection oven (GCA/Precision model 19; Fisher Scientific Co.) was used. It was preheated to approximately the desired maximum test temperature before each experiment. A calibrated mercury-glass thermometer was used to measure the oven temperature. The sample vial was situated in a 100-ml beaker, which supported and slowed the heating rate of the sample vial in order to accommodate the microwave oven temperature tracking.

Microwave oven. The microwave oven (model BPH-6000; Cober Electronics) used in this study is an industrial unit capable of up to 6 kW of microwave output power at 2,450 MHz. The maximum experimental power output was 4 kW. The oven was modified so that an external computer system could control the microwave power automatically. Inside the microwave oven, a styrofoam block (14 by 15 by 15 cm) contained one sample vial. The styrofoam provided the necessary thermal insulation to prevent the vial from losing too much heat to the environment (12).

Temperature probes. The model 2000A Fluoroptic thermometer (Luxtron, Inc.) measured the temperature of the glass vial during each experiment. This specialized instrument uses nonmetallic and microwave-transparent fiber optic probes. Transparent cellophane tape secured two probe tips, approximately 2 mm apart, to the outside surface of each glass vial opposite the spore inoculum. Temperatures collected from these two temperature probes were averaged for datum analysis, except for the data shown in Fig. 2, in which only one probe was used for temperature measurement. Since the thermal mass of the 0.7-mm-diameter probe is much lower than that of the glass vial, the probe and vial were in thermal equilibrium, a necessary condition for accurate temperature measurement. An external temperature reference (Kaye Instruments model HTR-300), a 100-Ω platinum resistance thermometer (Yellow Springs Instrument Co.) and a platinum resistance thermometer monitor (Kaye Instruments model 373) were used to calibrate each Fluoroptic probe before all experiments. The temperature measurements were accurate to ±0.5°C over the temperature range of interest (100 to 150°C).

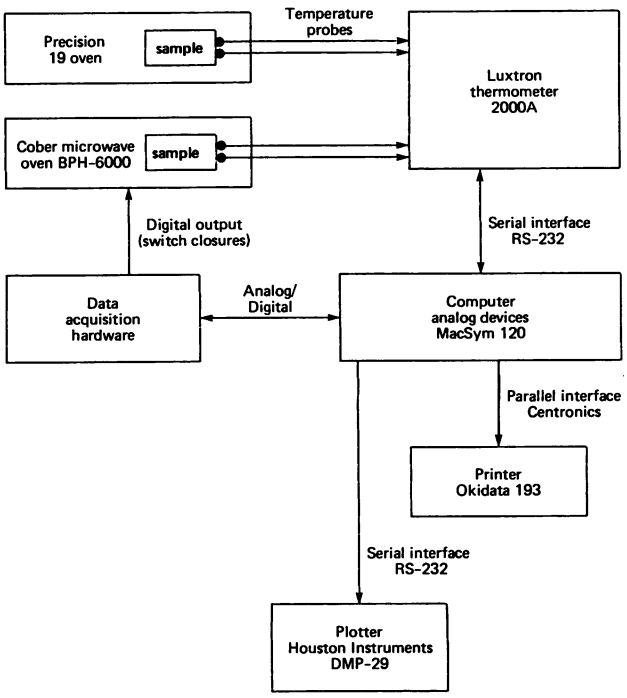

FIG. 1. System set-up for temperature control and datum acquisition.

Automation. A computer system (MacSym 120; Analog Devices) acquired data from the Luxtron thermometer for convenient datum printout. The computer also controlled the microwave generators. As the vial in the thermal oven heated via convection, the computer adjusted the microwave power in real time so that the vial in the microwave oven heated at the same rate as the vial in the thermal oven. The temperatures of samples in the convection and microwave vials tracked within 2°C. The entire experimental setup is shown in Fig. 1.

Exposure. To accurately control and measure the exposure temperature, only one pair of sample vials was exposed for each experiment, one vial in the microwave oven and the other in the convection oven. There were, therefore, slight temperature variations (less than 2°C from target temperatures) between tests. The exposure time is defined as the total time the dry-heat vial was situated in the convection dry-heat oven, regardless of temperature. Upon completion of exposure, both the dry-heat vial and the microwave vial were quickly removed from their respective ovens and cooled with a stream of filtered compressed air. Figure 2 shows a typical temperature-time profile of vials exposed to

both thermal and microwave treatments at various temperatures. Postcycle cooling is also shown.

The microwave power output varied from 0 to 4 kW during experiments. Since the objective of this study was only to determine the possible existence of nonthermal effects of microwaves on microorganisms but not the magnitude of such effects, no attempt was made to measure the microwave field strength or power in the vicinity of the sample.

RESULTS

Temperature profiles. The actual temperatures achieved in the experiments were 107, 117, 130, and 137°C. Figure 2 shows the representative comparative temperature profiles of both heat sources at 107, 117, 130, and 137°C. Except for a few time points at which the temperature of the microwave oven overshot during tracking, the computer temperature monitoring and measuring systems were able to minimize the temperature difference between the two heat sources to less than 2°C. The narrow range of the temperature variations between the two heat sources assured the validity of the results.

Sporicidal activities. Figure 3 shows the representative sporicidal kinetics of convection dry heat versus microwave heat at various temperatures. From these results, it is clear that temperatures below 117°C are not effective for dry-product sterilization because of a low lethal rate. At 130 and 137°C, the sample come-up time (the time required to reach maximum experimental temperatures) was approximately 20 min. Once the maximum experimental temperatures were reached, the spores were exponentially inactivated. The total exposure times to reduce 10^5 spores were 75 and 48 min at 130 and 137°C, respectively. There was no significant difference observed between convection and microwave heat in spore inactivation in all temperature ranges studied. The *D* values (the time needed to reduce the population by 90% at one temperature) at 117, 130, and 137°C were estimated to be 88, 14, and 7 min, respectively. The *Z* value (increase in temperature required for 90% reduction in *D* value) was estimated to be 18°C.

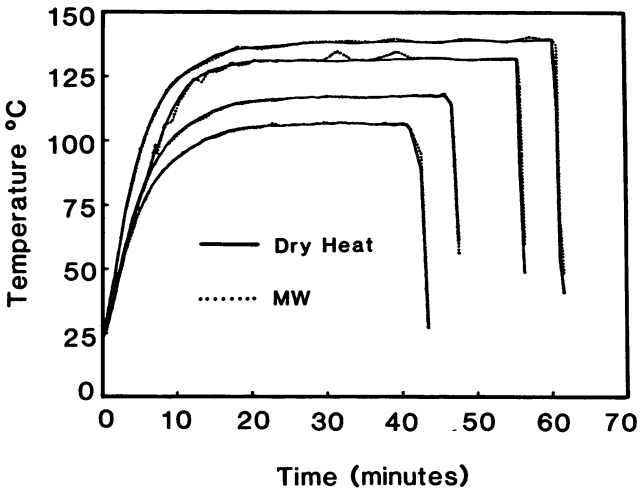

FIG. 2. Representative temperature-time profiles of convection oven- and microwave (MW) oven-heated samples. The microwave heating was designed to track the convection dry heat with minimum temperature variations.

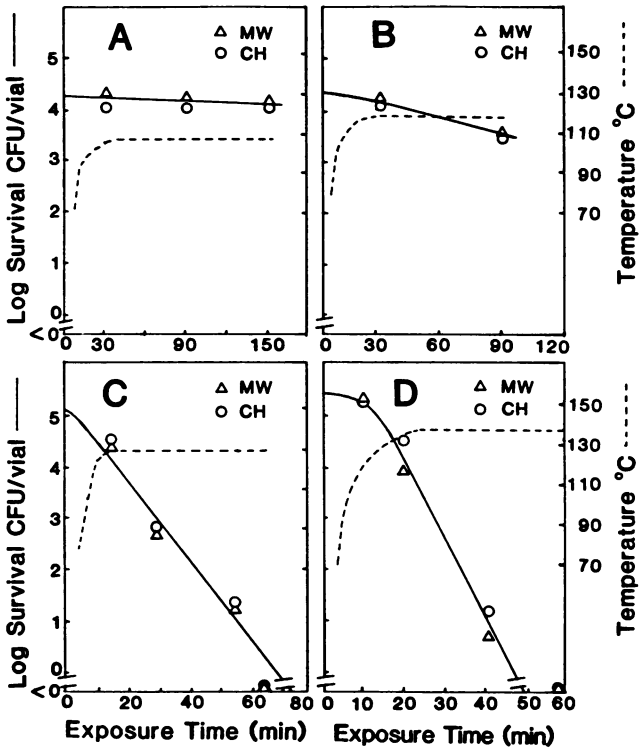

FIG. 3. Sporicidal kinetics of microwave (MW) versus convection dry heat (CH) at 107°C (A), 117°C (B), 130°C (C), and 137°C (D). The solid line is only for the purpose of visualization. The dotted line is an average temperature profile obtained from two temperature probes of the dry-heat sample of the longest exposure.

DISCUSSION

The development of a short cycle for microwave sterilization at relatively low temperatures was attempted under the assumption that the nonthermal effects of microwave energy may have an impact on sporicidal activities. In addition, the mechanism of the microwave sporicidal action was studied by comparing the spore inactivation kinetics from convection dry heat with those from microwaves. Under the experimental conditions, microwave heating at temperatures below 117°C was not effective for sterilization of dry medical devices because of a low lethal rate. Furthermore, within the temperature range studied, there was no significant difference in sporicidal activities between the microwave and convection thermal treatments. Both convection and microwave heating showed similar inactivation kinetics, provided that the microwave oven tracked the convection oven at nearly identical temperatures. These results strongly suggest that the sporicidal activities of microwave energy are merely a function of heat. No other factors seem significant enough to influence the sterilization process. Nonthermal effects are not significant in a dry microwave sterilization process.

Our experience in preliminary tests revealed difficulties in accurately tracking the microwave oven temperature to the convection oven (data not shown). The difficulties were especially true in experiments dealing with higher temperatures because the lethal rate increases exponentially as a function of temperature. At temperatures higher than 135°C, a slight temperature variation would cause a significant change in sporicidal activities. However, once the accurate temperature tracking was achieved, similar spore inactiva-

tion kinetics were repeatedly obtained from both heat sources. The temperature control, therefore, is the key to the experimental evaluation. Various observations found in the earlier literature (5, 12, 15, 23) (U.S. patent 3,753,651; Japanese patent 47860) were believed to be caused by variations in either temperature control or temperature measurement or both. With the aid of the computerized control system and Fluoroptic temperature probes, we have now unequivocally demonstrated that spore inactivation by microwaves in a dry state is caused solely by thermal effects.

The current study showed that a total dwelling time of at least 45 min was required to inactivate 10^5 inoculated dry spores at 137°C by both convection and microwave heating. The Z value of 18°C shows a slight variation from the published Z values of 17.5 (6), 21 (9), or 22°C (13) for still-air convection dry heat on *B. subtilis* subsp. *niger* spores and from the Z values of 21 (14) to 20°C (16) for dry-heat sterilization process validation. The sporicidal activities in the present studies are close to those found in the work of Wayland et al. (23). However, the thermal-electromagnetic interdependence found in their studies was not observed. In the study by Wayland et al., it is not clear why the sum of the interaction of microwaves and thermal heat was synergistic below 135°C but antagonistic at temperatures above 135°C. The experimental temperature variations in their study (±4 to ±9°C) could seriously affect the lethal rate (17) in the temperature range studied and thus the validity of their conclusion.

Extrapolation from the Z value in the current study shows that inactivation of 10^6 *B. subtilis* subsp. *niger* dry spores would require at least 26.4 min with a D value of 4.4 min at 140°C. These observations varied from the experimental results obtained by Lohman and Manique (12) in which 10^6 dry spores of the same strain were inactivated by microwaves in less than 3 min cycle time with a constantly rising temperature up to 140°C. In their study, multiple vials were used for microwave treatment. A gradient of the spore inactivation rate among vials situated in different locations was observed, presumably because of heterogeneous heat loss. Within a temperature range of 130 to 140°C, the D value from their work is approximately 0.1 min. These results drastically disagree with the observed D value of 8 min at 137°C and the calculated D value of 4.4 min at 140°C from the current studies.

The heat distribution in vials during microwave treatment was found to be surprisingly uneven. On the same insulated vial, a temperature variation of 4 to 5°C was detected between a distance of only 1 to 2 cm (data not shown). Temperature probes, therefore, must be located at the exact location where the spores are inoculated in order to obtain accurate data. The method of temperature measurement used by Lohman and Manique (12) required an opening of the microwave oven door and removal of the insulated materials. Samples in a microwave oven undergo a rapid decrease in temperature when the oven door is opened. The temperature recorded by the infrared method used by Lohman and Manique after these procedures may therefore significantly underestimate the true temperature of the samples.

In dry-heat sterilization cycle development, the mass of the glassware being sterilized essentially determines the length of the come-up time. In general practice, to sterilize an industrial-scale load of glassware by convection dry heat in an overkill approach requires a dwelling time of 2 to 3 h to establish a safety assurance level of 10^{-6} at 160°C. The exposure cycle would be considerably longer if the come-up time and heat distribution issues were considered. In our microwave heating system with a constant power output of 4 kW and a single-vial load, the maximum rate of temperature increase from 22 to 140°C is 5 min (data not shown), compared with a minimum of 20 min required for the convection dry-heat oven with the same load. A larger load would further increase the difference in the come-up time between these two methods. It seems, therefore, that the only advantage to microwave sterilization of dry products lies in a shorter come-up time. Once the sterilization maximum temperature is obtained, there are no advantages in sporicidal activities to a microwave sterilization process.

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