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DISINFECTION OF WASTEWATER BY MICROWAVES.(U)
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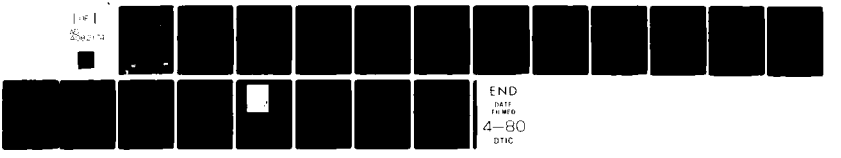

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Results from a laboratory study show that microwave energy can be used for disinfection of wastewater. The time required for destruction of bacteria by microwaves was reduced over that of conventional heating. Destruction of wastewater bacteria and a cell-suspension of *E. Coli* B. was logarithmic after an initial lag phase, which was dependent upon the volume used. Thermophilic *B. stearothermophilus* cells were used to try to determine if the mechanism of destruction was thermal. The microwave oven was set at optimum growth

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temperature of this species (60°C). Destruction was much more rapid in the microwave oven than in a 60°C waterbath. More than 90% of the cells were destroyed by heating 30 minutes in a microwave oven, although continued heating had little further effect. Repeated microwave treatment for 1-hour periods also did not increase the number of cells destroyed. It was thought that by using chilled cells prior to heating or chilled buffer for dilutions after heating, increased thermal shock might be observed. However, neither the temperature of the cells before treatment with microwaves nor the temperature of the diluent buffer showed any effect on the rate and extent of bacterial destruction.

Preface

This report was prepared by Dr. I.K. Iskandar, Research Chemist, L.V. Parker, Microbiologist, and K. Madore, Physical Science Technician, of the Earth Sciences Branch, Research Division, U.S. Army Cold Regions Research and Engineering Laboratory, and Dr. C. Gray, Chairman, Microbiology Department, Medical School, Dartmouth College, and Dr. M. Kumai, Research Physicist, Physical Sciences Branch, Research Division, CRREL.

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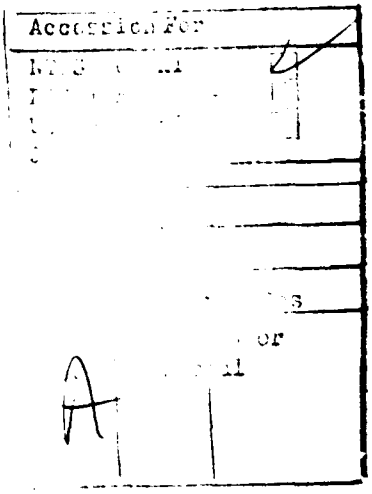

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DISINFECTION OF WASTEWATER BY MICROWAVES

I.K. Iskandar, L. Parker, K. Madore,
C. Gray and M. Kumai

Introduction

The major objective of wastewater disinfection is to produce a finished effluent that is acceptable from a public health standpoint. Several methods have been proposed and used to disinfect wastewater and sludge. Over the past 70 years chlorination has evolved as the commonly used method of disinfection for water supplies and wastewater in the United States. However, during the past 20 years, increasing attention has been given to the environmental impact of chlorinated compounds. The various chlorinated hydrocarbon pesticides, such as DDT, elicited much of the early concern, but recently an increasing number of laboratory and field investigations have documented the potential toxic effects associated with the chlorination of wastewater. Among these were the studies by Arthur et al. (1975), Arthur and Eaton (1971), Esvelt et al. (1973), and Zillich (1972). The literature on residual chlorine toxicity to aquatic life was reviewed by Brungs (1973 and 1976). Dechlorination has been used to solve the potential problem of residual toxicity of chlorinated effluent to aquatic life; however, it is costly and time-consuming.

Ozone is commonly used in Europe to disinfect water supplies and is a stronger and faster oxidizing agent than chlorine (Rosen 1973, Layton 1972). Several reports have appeared on the superiority of ozone over chlorine in destroying bacteria and viruses (Fetner and Ingols 1956, Smith and Bodkin 1944, Nebel et al. 1973), and on the ability of ozone to reduce color, odor, oxygen demand, and turbidity of wastewater (Nebel et al. 1973, Evans 1972, Greening 1974). While some investigators (Rosen 1973, Harr 1975) believe that the byproducts of wastewater ozonation lack the potential toxicity of the byproducts of chlorination, some studies (Arthur et al. 1975, MacLean et al. 1973, Rosenlund 1974, Ward 1976) indicate that undesirable biological effects could possibly be associated with the ozonation of wastewater. In addition, the method is costly and can be unreliable with poor quality effluents. Recently, the use of bromine for disinfection has received much attention because of its greater effectiveness and lower cost compared with chlorination (Wilson 1974). Mills (1973a, 1973b) concluded that compared to chlorination, chlorobromination produces a better destruction of viruses and bacteria and a reduced residual toxicity.

Several other methods have been used or proposed for disinfection of sludges. These include treatment with ultraviolet light, γ-radiation and electron beams. The problem with the use of ultraviolet light in disinfection of wastewater and sludges is its limited penetration, which

requires treating the wastewater in a thin film. Since the use of γ-radiation or an electron beam requires the availability of a reactor near the site, these methods have limited application.

An alternative method for disinfecting wastewater is by microwave radiation; however, the mechanism of bacterial destruction by microwave radiation is not well understood. The purpose of the research described in this report was to investigate the rate and extent of disinfection and possible mechanism(s) of bacterial destruction by microwave radiation.

Literature Review

Microwave energy generates heat directly inside a material which permits higher heat fluxes and thus a much faster temperature rise than in conventional heating. In conventional heating, heat is transferred to the surface of the material to be heated by conduction, convection, and/or radiation.

While microwave energy is recognized to have bactericidal ability when heating to lethal temperatures is allowed, it has not been resolved whether microwave energy has a nonthermal bactericidal effect. Among the researchers who have evidence of strictly thermal destruction of bacteria, yeast, or viruses by microwave energy are Goldblith and Wang (1967), Lechowich et al. (1969), Corelli et al. (1977), and Walker et al. (1974). These experiments were all conducted in the 2.4 to 4.0-GHz range, but the power varied depending on whether heating was allowed or not.

There are several theories proposed explaining possible nonthermal destruction of microorganisms by microwave energy. Carroll and Lopez (1969) listed three possible mechanisms. The first method of destruction proposed is that heat is generated in the cell faster than in the surrounding medium. Therefore the cells reach a much higher temperature than the surrounding medium. The second method proposed is mechanical destruction: since most microbial cells bear a charge (usually negative) the cell may be disrupted by rapid oscillations caused by the high frequency microwave field. The third method proposed is that the energy of a given frequency is selectively absorbed by critical organic molecules, such as essential protein or DNA, and these molecules are irreversibly denatured.

Ingram and Page (1953) and Stack (1974) have done calculations on whether there can be a large temperature difference between the cells and suspending medium, and Page and Martin (1978) have observed the death rates of cell suspensions vs dried films of cells. At the present, there is no general agreement on this question.

Webb and Booth (1969) have found evidence that frequencies in the 65 to 75-GHz region were selectively absorbed by the cell's DNA, RNA, and protein molecules, directly affecting metabolic activity and growth. Averbeck et al. (1976) confirmed that microwave energy in this range did affect growth. However, while they were able to prove that DNA absorbs microwave energy at these frequencies, microwaves did not have a mutagenic effect on the DNA in the bacterial and yeast cells tested.

Other biological effects have been reported but are not well supported with data. Cope (1976) felt that superconduction in biological systems offered a reasonable physical basis for possible nonthermal effects of microwaves. Fröhlich (1968) proposed that specific microwave frequencies might have an effect on the activity of various biological substances. He based this theory on the opposite charges and varying sizes of many biological membranes that might allow longitudinal vibrations. Olsen et al. (1966) proposed that pearl chain formation may allow microorganisms to clump and present a better target for microwaves. Heller et al. (1963) observed that pearl chain formation in a colloidal suspension was due to loss of zeta potential induced by radio frequency waves (1-100 MHz).

In summary, there currently appears to be some agreement among researchers on the effects of microwaves on living microorganisms and their biological processes. While no one has been able to prove a lethal nonthermal effect of microwaves, there is evidence that specific frequencies are absorbed and can alter the cell's metabolism. Microwave energy in the 65 to 75-GHz range has been shown to be selectively absorbed by DNA, RNA and protein molecules and this affects metabolic activity and growth. It is believed at this time that this absorption does not lead to denaturation or mutation of the DNA molecule. The mechanisms of any nonthermal effects have yet to be proven. It should be stressed that much more detailed work needs to be done before there is general agreement on the effects of microwave energy on microorganisms.

Materials and Methods

A conventional microwave oven (Amana model RR-10 or Sears Model 99651) was used for all the experiments. All samples were placed in the center of the oven and heated with maximum power -- 750 watts for the Amana microwave oven and 600 watts for the Sears model. A 250-ml beaker was used to contain the samples.

Different volumes of undisinfected secondary wastewater were tested to determine the survival curves. After these samples were removed from the oven they were covered with plastic wrap and allowed to cool prior to dilution. Fecal coliforms in the wastewater were enumerated by using

the fecal coliform membrane filter technique described in the 14th edition of Standard Methods for the Examination of Water and Wastewater (APHA 1976). All samples were done in duplicate. The temperatures of the samples were taken every 10 s to determine the heating curve.

Cell suspensions of Escherchia coli B were tested to determine the survival curve for this organism when exposed to microwave heating at 60°C. The cells were grown in Trypticase Soy Broth with aeration at 37°C. They were then centrifuged at 10,000 rpm, resuspended in an equal volume of 0.01 M potassium phosphate buffer (pH 7.0), centrifuged, and resuspended in the same volume buffer. The dilution blanks were also 0.01 M PO_4 buffer. Fifty-milliliter samples were heated in the microwave oven which was temperature programed for 60°C. The Amana oven is programable in 10°F (5.5°C) increments and uses a shielded temperature probe to determine temperature. Pour plates of Trypticase Soy Agar incubated at 37°C were used to enumerate the samples. Triplicate plates were made for each dilution.

A similar procedure was used on cells of Bacillus stearothermophilus, a thermophilic bacterium. These bacteria were used in the experiment to test whether the bactericidal mechanism of the microwaves resulted from heat or other effects. The beakers had to be covered with plastic wrap during the prolonged heating that was necessary for destruction of this organism, or otherwise total evaporation of the sample would have occurred. Incubation for these thermophilic bacteria was at 55 °C rather than 37°C.

Experiments to determine the survival curve for Bacillus stearothermophilu in a water bath were done by placing 50 ml of the cell suspension into a sterile double-necked flask which was then placed in a 60°C water bath. The temperature of these cells was determined with a thermometer placed in one of the two necks of the flask.

Later experiments involved heating and chilling the cell suspension and dilution blanks to investigate the effects of temperature on survival.

Following the experiments, the samples were examined with transmission and scanning electron microscopes to determine if treatment with microwaves caused any morphological change in the bacterial cells. In these examinations, 4 µl of each sample was placed on a filmed grid, dried in a desiccator, shadowed with chromium vapor in a vacuum chamber at an angle of 19.5°, and then examined by the transmission electron microscope. For scanning electron microscopic work, the samples were coated with gold vapor to a thickness of 100 Å by conventional shadowing techniques.

Results and Discussion

The first experiment used 100 ml of undisinfected secondary wastewater in a 250-ml glass beaker placed in the microwave oven (Sears Microwave

Oven Model 99651) for a variable period of time. Fecal coliform bacteria were enumerated on samples (similar aliquots) after 30, 40, 50, 60 and 70 s exposure in the microwave oven. Figure 1 shows the number of living organisms as a function of time. It is evident that a lag phase exists, which in this experiment is 30 s after which the death increases logarithmically. Experiments were then conducted with from 15 to 100 ml of wastewater. Figures 2, 3, and 4 show that the lag phase is dependent on the volume of wastewater used, being longer with the larger volume. Also the time needed for complete destruction of coliform bacteria is dependent on the volume used. These data show that microwave energy can successfully be used to destroy fecal coliform bacteria present in wastewater.

Preliminary experiments were then conducted to investigate the mechanism(s) of disinfection. Figure 5 shows the change in temperature of different volumes heated in a microwave oven with time. By correlating the length of the lag time before destruction by microwaves with the temperature data, it appears that destruction of the microorganisms did not begin until after the water temperature reached 40°C. Therefore, the factors affecting the time necessary for destruction appear to include the sample volume and temperature.

Geopfert et al. (1969) studied the heat resistance of E. coli in different environments. The D value (time required to destroy one log of the number of bacteria) for E. coli at 57.5°C was 0.8-1.5 minutes. In the present study, the D value at this temperature (Fig. 4) is much less (0.33 min). This result indicates that microwave energy may have a nonthermal lethal effect, although the differences may be solely attributable to the more rapid heating that is possible with microwave energy.

Preliminary tests for the mechanism(s) involved the use of the transmission electron microscope. However, it was difficult to conclude the reason for destruction from these experiments due to the presence of so many types of bacteria in the wastewater and the nature of the transmission electron micrographs (data not included).

Experiments were then conducted on a pure culture of E. coli B. Figure 6 shows the survival curve of this organism when heated in a microwave oven temperature programed to hold at 60°C. This species was selected because it is one of the indicators used for fecal contamination of water. This explains the similarity between the D values for equal volumes of this culture (Fig. 6) and the fecal coliforms in the wastewater (Fig. 3).

To investigate the effect of microwave radiation on a thermophilic bacterium, a pure culture of B. stearothermophilus ATCC 12980 was obtained. The cell suspension was heated in a microwave oven that was temperature programed for 60°C. A one log reduction, equivalent to 90% destruction in the number of survivors, occurred in the first 30 minutes (Fig. 7).

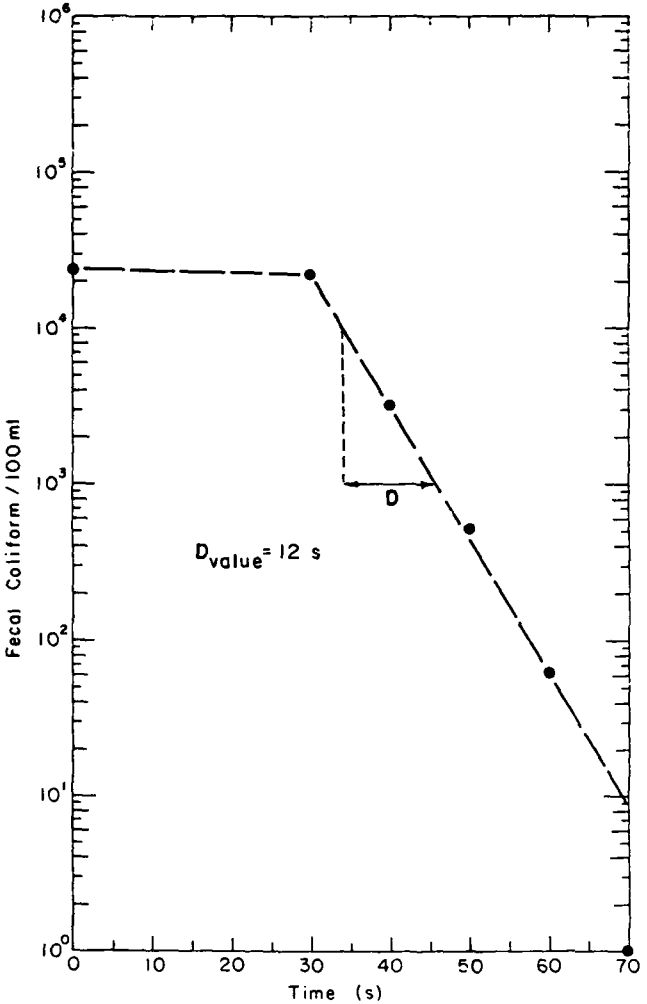

Figure 1. Exposure of 100 ml of wastewater to microwave heating.

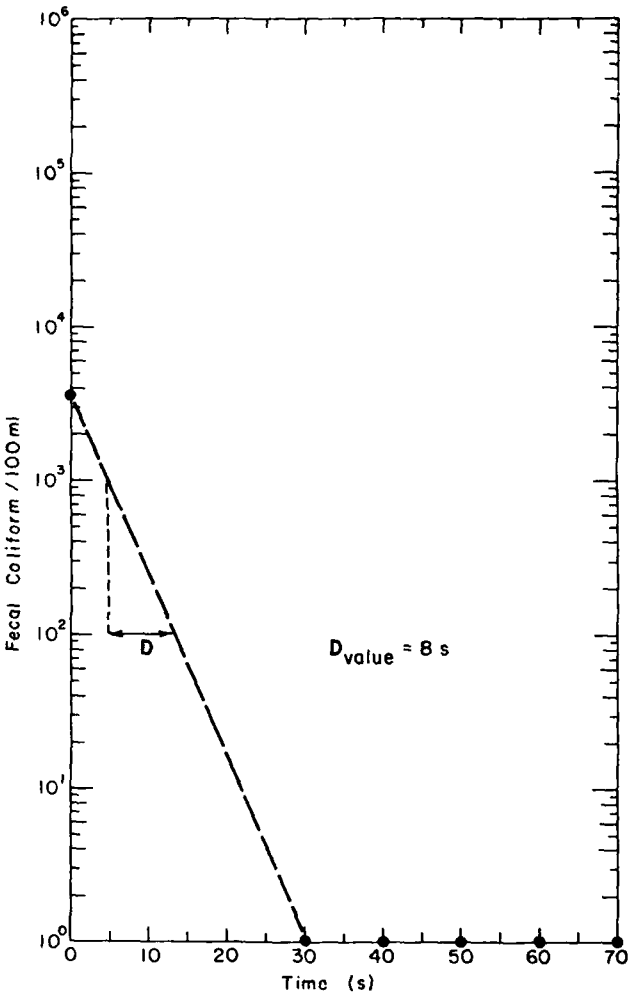

Figure 2. Exposure of 15 ml of wastewater to microwave heating.

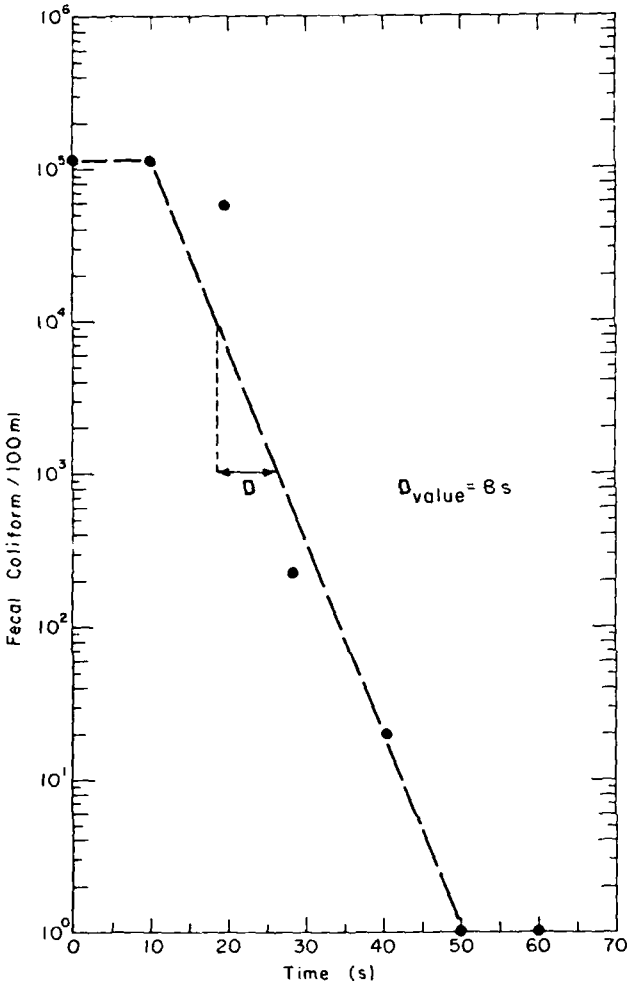

Figure 3. Exposure of 50 ml of wastewater to microwave heating.

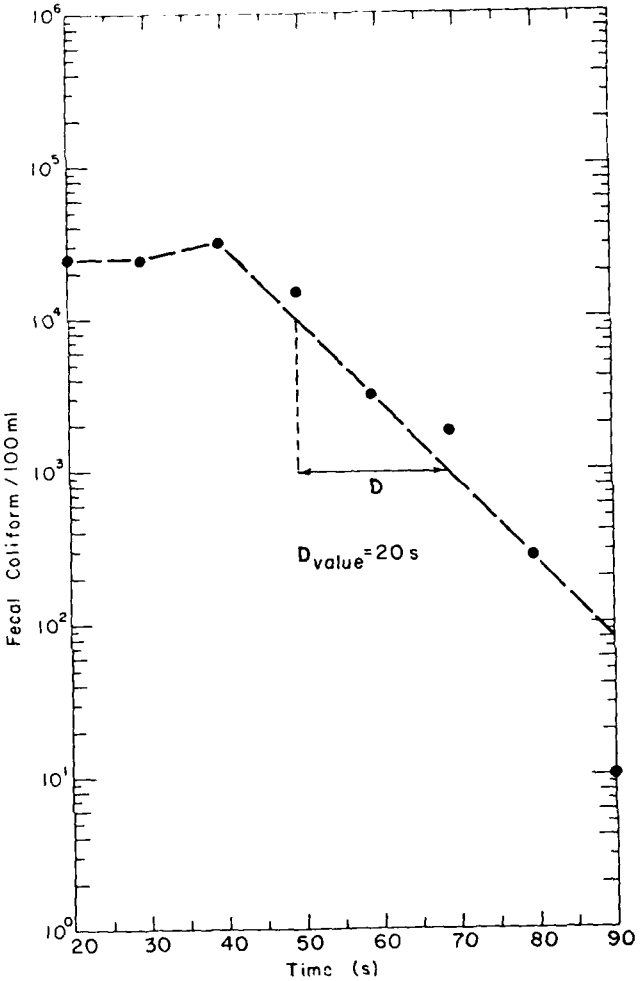

Figure 4. Exposure of 200 ml of wastewater to microwave heating.

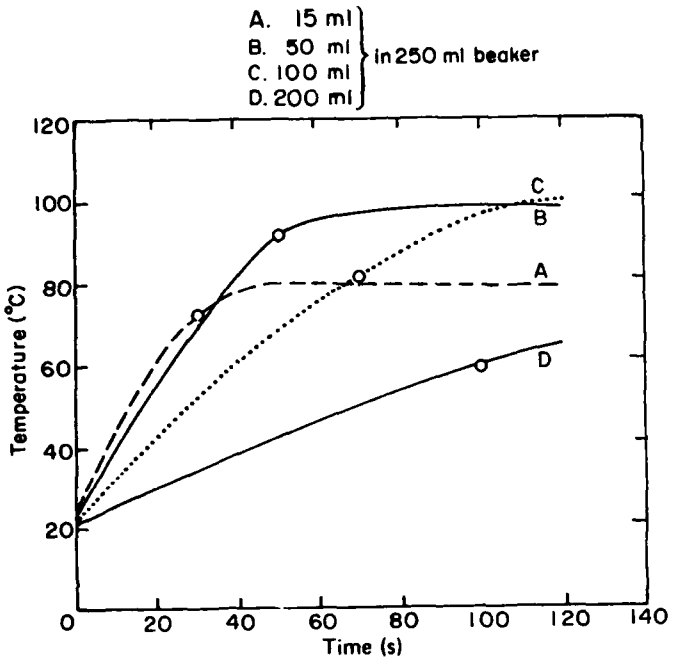

Figure 5. Heating curves for different volumes of wastewater in the microwave oven. Open circles indicate the time and temperature required for 99% kill of microorganisms.

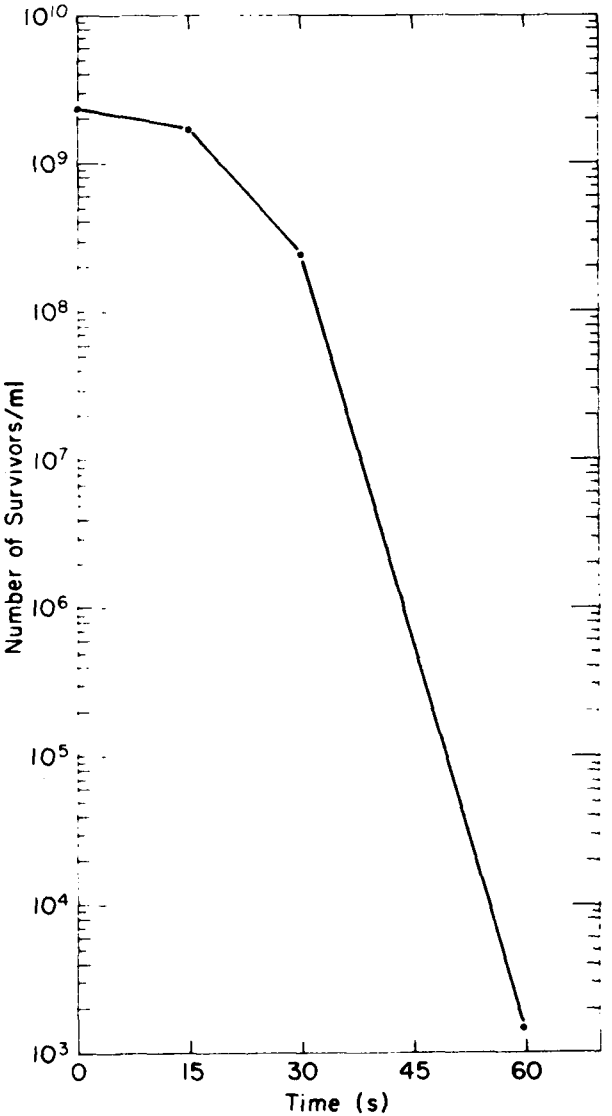

Figure 6. Survivor curve for a 50-ml suspension of E. coli B cells heated in a microwave oven temperature programed for 60°C.

Only slight destruction occurred after the initial 30 minutes, although samples were subjected to a total of 2 hours of treatment. Because the optimum growth temperature of this species is 50-65°C, destruction at this temperature was not anticipated.

In the following experiment aliquots of the same culture were subjected to conventional water bath or microwave heating at 60°C. Figure 8 shows the results obtained from the microwave and control (water bath) treatments at 60°C. Destruction of the microorganisms in the microwave oven was very rapid initially, then decreased and almost leveled off after 30 min. Approximately 80% of the bacteria were destroyed in the first 5 min and an additional 10% were destroyed during the following 25 min. Eighty-percent destruction of the population in a water bath required heating for 2 hours (Fig. 8). While the rate of destruction was greater with microwave heating, the total log reduction was nearly the same for the two types of heating. Interpretation of this experiment is difficult because the rate of heating is much faster in a microwave oven and the temperature control in the microwave oven was poor.

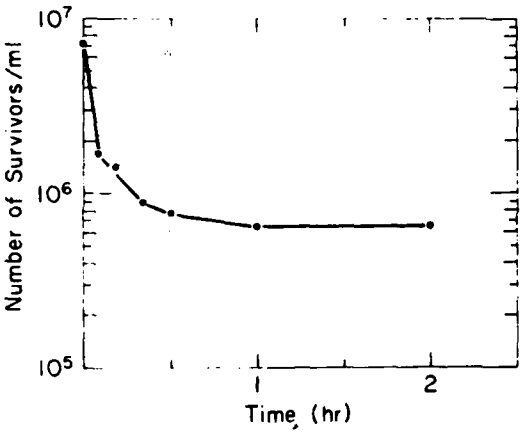

Figure 7. Survivor curve for B. stearothermophilus (ATCC 12980) cells heated in a microwave oven temperature programed for 60°C.

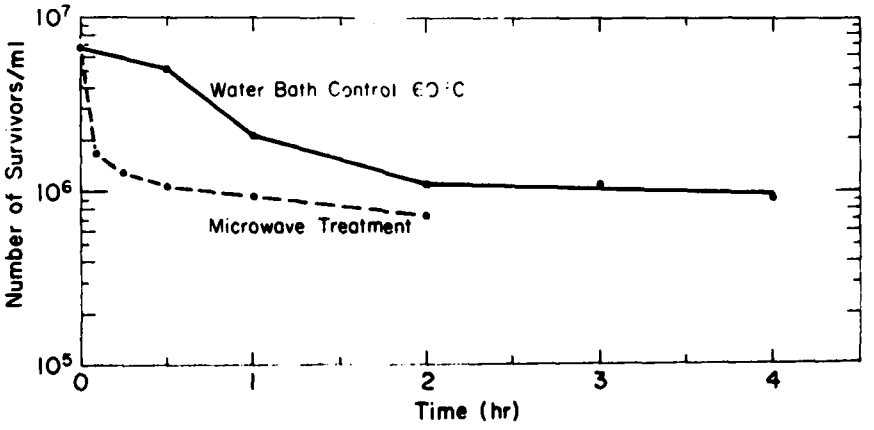

Figure 8. Survivor curve for B. stearothermophilus (ATCC 12980). Cells were heated in a microwave oven temperature programed for 60°C and in a 60°C water bath.

To investigate the effect of repeated exposure to microwave radiation on the destruction of bacteria, B. stearothermophilus cells were heated in a microwave oven (programed for 60°C) for one hour, taken out and allowed to cool to room temperature (20-22°C), and then heated again for one hour. This treatment was repeated three times. Figure 9 summarizes the results obtained. There was no significant reduction in the number of bacterial survivors after the initial few minutes. About 80-90% of the bacteria were destroyed during the first few minutes of exposure to microwaves followed by very little or no effect on the survivors. Spore stains of the cell suspensions prior to and after heating did not indicate that there was a large enough proportion of spores to account for the residual resistance that was observed. However, this explanation should not be totally eliminated at this time. Another explanation may be that the cells in one phase of growth may be more resistant to this destruction.

Table 1 shows the effect of the temperature of the cell suspension prior to microwave treatment on the survival of E. coli B cells subjected to microwave treatment. It seems that the temperature of the cell suspension prior to treatment has no consistent relationship with the survival of E. coli B cells heated in a microwave oven. Inconsistent heating may have been the cause of the erratic nature of the data. Table 2 shows the effect of the temperature of diluent after microwave treatment on the survival of B. stearothermophilus cells. Again temperature of the diluent had no clear effect on the survival of this type of bacteria. These last three experiments indicate that temperature shock is probably not the mechanism involved in the cells' destruction.

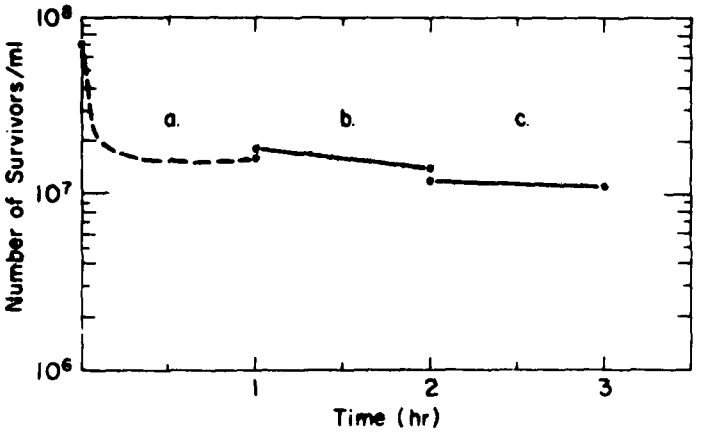

Figure 9. Survivor curve for B. stearothermophilus ATTC 12980 cells subjected to repeated 1-hr doses of microwave heating. a = first 1-hr dose of microwave energy, b = second 1-hr dose of microwave energy, and c = third 1-hr dose of microwave energy.

Table 1. Effect of the temperature of the cell suspension on the survival of E. coli B cells subjected to microwave treatment.

Heating time (s)	No. survivors/ml: Cell suspension at 23°C	Cell suspension at 6°C
Trial A		
0	1.9×10^8	1.9×10^8
15	2.4×10^8	2.9×10^8
30	7.8×10^7	1.5×10^8
45	$< 3 \times 10^6$	$< 3 \times 10^6$
60	$< 3 \times 10^5$	$< 4.2 \times 10^2$
90	$< 3 \times 10^5$	$< 3 \times 10^5$
Trial B		
0	5.8×10^7	1.1×10^8
15	6.7×10^7	1.3×10^8
30	1.9×10^7	1.5×10^6
45	$< 3 \times 10^4$	1.4×10^7
60	1.1×10^6	4.0×10^6
90	$< 3 \times 10^4$	1.5×10^6

Table 2. Effect of the temperature of diluent upon the survival of microwave-treated B. stearothermophilus ATCC 12980 cells.

	No. of survivors/ml: Sample 1	Sample 2
Initial Cell Density	6.9×10^6	5.7×10^6
Temperature of diluent		
Buffer at 23°C	3.4×10^6	2.9×10^5
Buffer at 55°C	2.6×10^6	2.7×10^6
Buffer at 6°C	4.6×10^6	2.5×10^6

Figure 10. Scanning electron micrograph of Bacillus stearothermophilus prior to microwave treatment.

Prints were also taken by the scanning electron microscope of cell suspensions of B. stearothermophilus exposed to microwaves. Unfortunately the concentrations of cells selected tor electron microscopic examination after microwave treatment were not in the proper range and therefore no useful micrographs were taken. Figure 10 is a scanning electron micrograph taken prior to treatment. This type of electron microscopy clearly delineates the surface structure of the bacteria and should prove useful in any further work.

Summary and Conclusions

Disregarding the cost of operation, microwaves can be used to disinfect wastewater. The time required for disinfection is much less than with conventional heating methods. There is logarithmic death of coliform bacteria after a lag phase. The length of the lag phase depends on the type of bacteria, the volume to be disinfected and the contents of solution.

Further studies should be more quantitative with better temperature control in order to determine whether the more rapid rate of destruction with microwave heating, as compared with conventional heating, is due to microwave energy per se, rapid heating in the microwave oven, or "hot spots" due to poor temperature control in the microwave oven. Although no conclusions could be drawn from the limited use made of the electron microscopes, the scanning electron microscope should prove very useful in any further study of the effect of microwaves on bacterial cell walls.

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