Journal of Applied Biological Sciences 2 (1): 67-71, 2008
ISSN: 1307-1130, www.nobel.gen.tr

Effect of Microwaves on Some Gram Negative and Gram Positive Bacteria

Serap GEDIKLI[1] Özge TABAK[1] Özlem TOMSUK[2] Ahmet ÇABUK[2,*]

[1] Osmangazi University, Graduate School of Natural and Applied Sciences, Eskişehir, TÜRKİYE

[2] Osmangazi University, Science and Art Faculty, Department of Biology, Eskişehir, TÜRKİYE

[*] Corresponding Author
e-mail: acabuk@ogu.edu.tr

Received: May 28, 2007
Accepted: July 11, 2007

Abstract

In this study, suspension of *Escherichia coli* ATCC 25922, *Bacillus cereus* NRRL 3711 and *Staphylococcus aureus* ATCC 25923 were exposed to microwave. The degrees of inactivation by the various time, initial bacterial cells amount and different power of microwave were compared systematically. Maximum efficiency of microwave was observed at 60 sec exposure time, 1.0 x 10^8 initial bacterial cells, and full power (900 W) for *E.coli* cells. Experimental data shows that microwaves apparently produced lethal effects on the examined bacteria by heat generated during microwave exposure. Water activity was not changed microwave efficiency for the each examined bacteria under the determined optimum conditions.

Key words: microwave, *Escherichia coli, Bacillus cereus, Staphylococcus aureus*

INTRODUCTION

There have been several publications in recent years which have mentioned possible lethal effect of microwave on microbial species especially bacteria [1,3,4]. The microwave bands called UHF (300 to 3.000 MHz) are used in numerous commercial devices, including television, microwave communications, microwave ovens, medical diathermy, radar technology, and an abundance of special equipment designed for specific uses [1]. Nowadays, also, the use of microwave radiation has become popular in the industrial applications particularly food and related industry [2,3]. Despite many studies on microbial damage by microwave radiation, the mechanism of destruction is not entirely understood. Several investigators have already observed the damage of microorganisms subjected to a microwave field was due to thermal effects [1,4]. Microwave heating is known to inactivate many microorganisms for instance, *Escherichia coli*, *Streptococcus faecalis*, *Staphyloccocus aureus*, *Bacillus subtilis* spores, *Salmonella* sp., *Lactobacillus plantarum*, *Listeria* spp., *Saccharomyces cerevisiae* and *Clostridium perfringens* [3-10]. However, some publications have attempted to ascertain if such radiation has a non thermal effect on microbial burden. According to Olsen (1965) conidia of *Aspergillus niger* and *Penicillium* sp. were inactivated by non thermal effects of microwave radiation [11].

This paper reports investigation on the effect of microwave on the viability of some gram negative and gram positive bacteria including *Escherichia coli*, *Bacillus cereus* and *Staphylococcus aureus*. Also, possible nucleic acids released in supernatant due to microwave effect on the cell wall and effect of water activity was investigated under the determined optimum conditions.

MATERIALS AND METHODS

Escherichia coli ATCC 25922, *Bacillus cereus* NRRL 3711, *Staphylococcus aureus* ATCC 25923, were selected as model microorganisms for determine inactivation effect of microwave in this study. The pure bacterial strain was incubated at 37 °C for 24 h in the Nutrient Broth (Merck) at neutral pH. Serial dilutions were prepared in 9 ml of 0.85 % (w/v) sterile saline solution and final density was adjusted to MacFarland 0.5. The experiment was performed in various times, different initial bacterial concentration and different power (90, 360 and full power 900) to determine the effect of microwave radiation, achieving colony counting methods. For the microwave application, a 2,450 MHz microwave oven (Bosch) was used. The glass tube containing 10 ml of cell suspension were placed in the center of the oven and exposed to microwaves. After the microwave treatment, each tubes containing bacteria were diluted with sterile saline solution and strirred for suspension of cells. After serial dilutions, bacteria in the sterile saline were spread over a standart agar plate (Nutrient Agar, Merck). The plates were incubated at 37 °C for 24 h and colonies was counted after incubation period. Also, untreated samples were used as a control group of the each tested microorganisms.

To determine the effect of the viability of bacterial cells on the microwave efficiency on the viable cells amounts of bacteria were prepared using MacFarland from 0.5 to 2 for 60 sec.

In order to determination of the possible nucleic acid in the supernatant via microwave effect on the cell wall. The amount of released from the microwave treated cells was measured at 260 nm using a UV-visible spectrophotometer (Jasco V-530).

After the cells were treated by microwave radiation the shape of cells was examined by microscopic observation for the screening of microwave damage on cell burden. The bacteria used in this study were staning with crystal violet (1 min) for the purpose of microscopic observation. In addition, effect of water activity (a_w) on the microwave damage were carried out aseptically with a serious tubes containing glycerol among 0,1-5,0 % for each bacteria. All the experimental study were carried out aseptic conditions.

RESULTS

The inactivation patterns of the microwave radiated cells were investigated using cell suspensions of *E. coli*, *B. cereus* and *S. aureus* (Fig 1).

Figure 2 was shown that changes in temperature with microwave heating processes for the each examined bacteria. The effects of the initial bacterial cells concentrations on the microwave efficiency with power of 360 on the viability of cells were investigated and related results were presented in Figure 3.

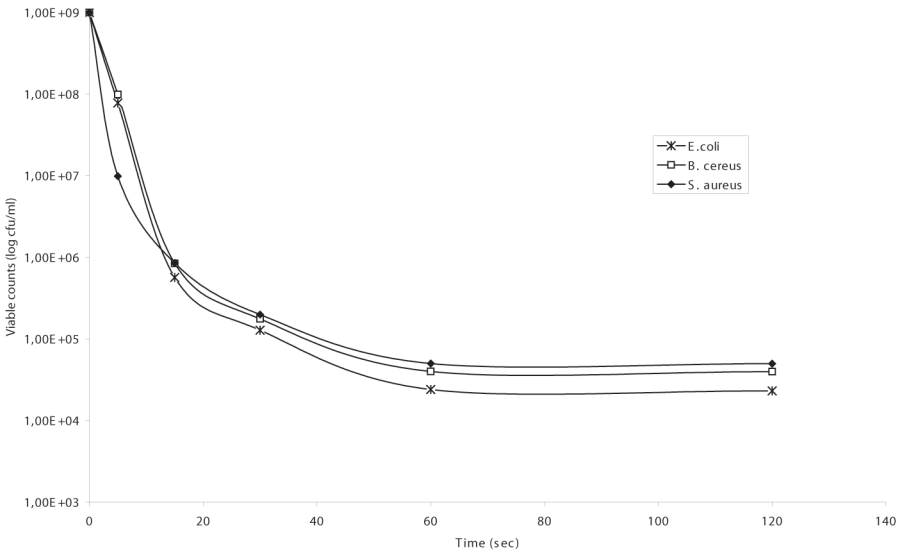

Figure 1. Change in the viable count of the microwave radiated cells of *E. coli*, *B. cereus* and *S. aureus*.

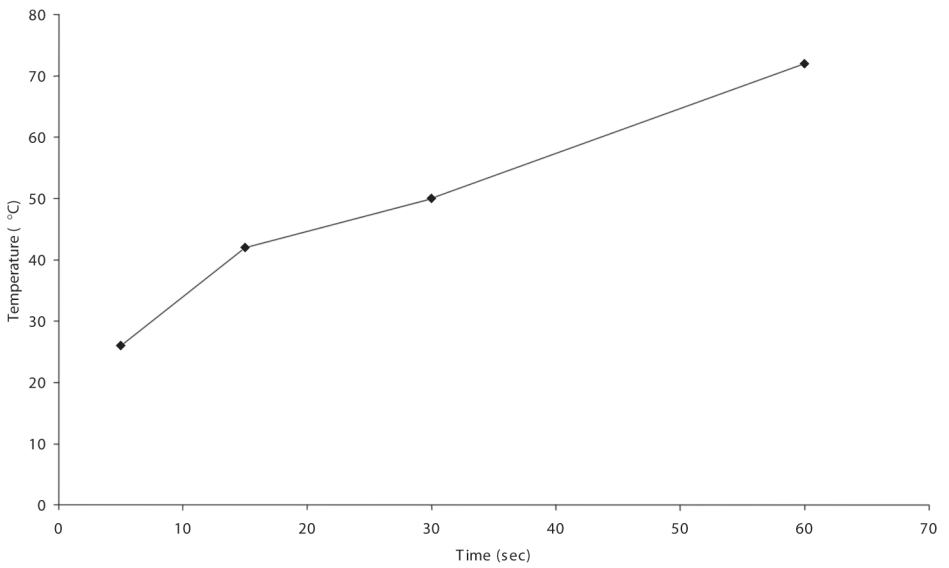

Figure 2. Change in the temperature of bacterial cell suspensions relative to microwave exposure time.

The effect of power was investigated in the range of 90, 360 and full power 900 and the results were presented in Figure 4.

Also, Figure 5 was shown that nucleic acid released into the cell suspension of each bacterium from microwave-radiated bacterial cells relative to the time by microwave radiation. *Escherichia coli* cells were found to be more sensitive bacteria to microwave radiation. Therefore, microwave treated and untreated *E. coli* cells were monitored with light microscope (Fig 6). In addition, the water activity ranged from 0,994 to 0,752 was not changed microwave efficiency for the each examined bacteria under the determined optimum conditions.

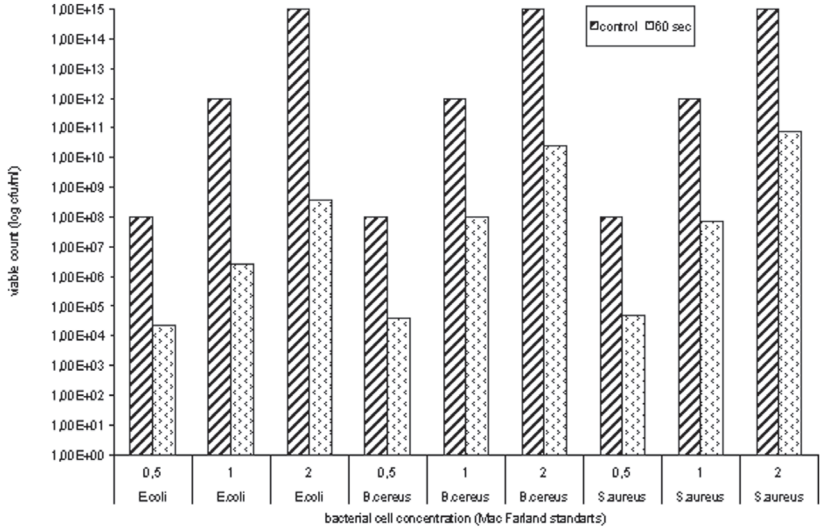

Figure 3. Effect of initial bacterial concentration on the microwave damage on viable cells of *E.coli*, *B. cereus* and *S. aureus*.

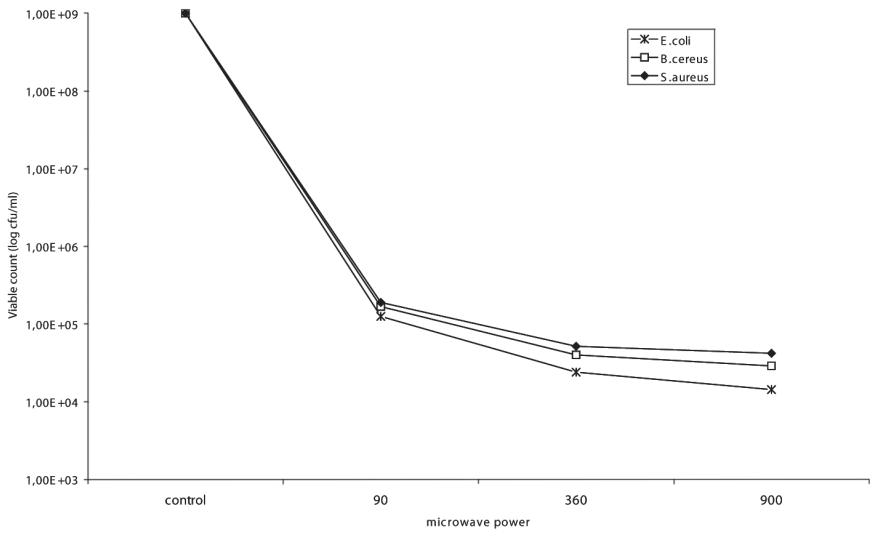

Figure 4. Effect of the different microwave power on the viable counts of *E. coli*, *B. cereus* and *S. aureus*.

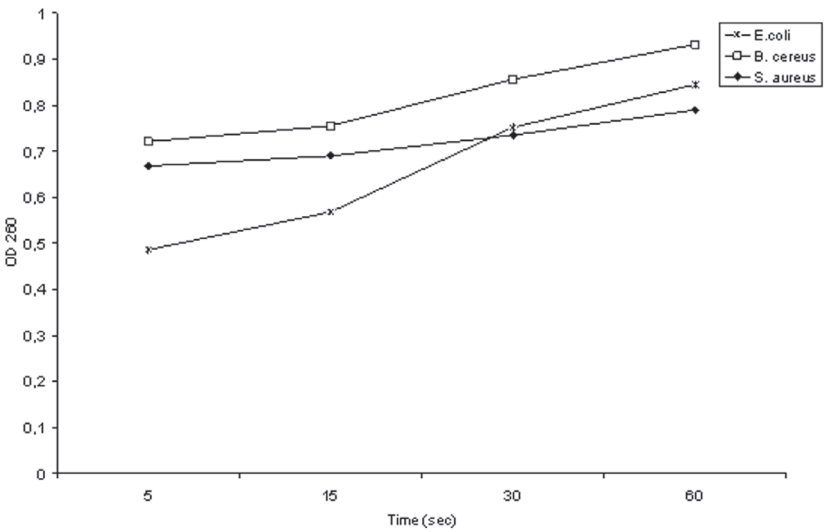

Figure 5. Nucleic acid released into the cell suspension of each bacterium from microwave-radiated bacterial cells relative to the time by microwave radiation.

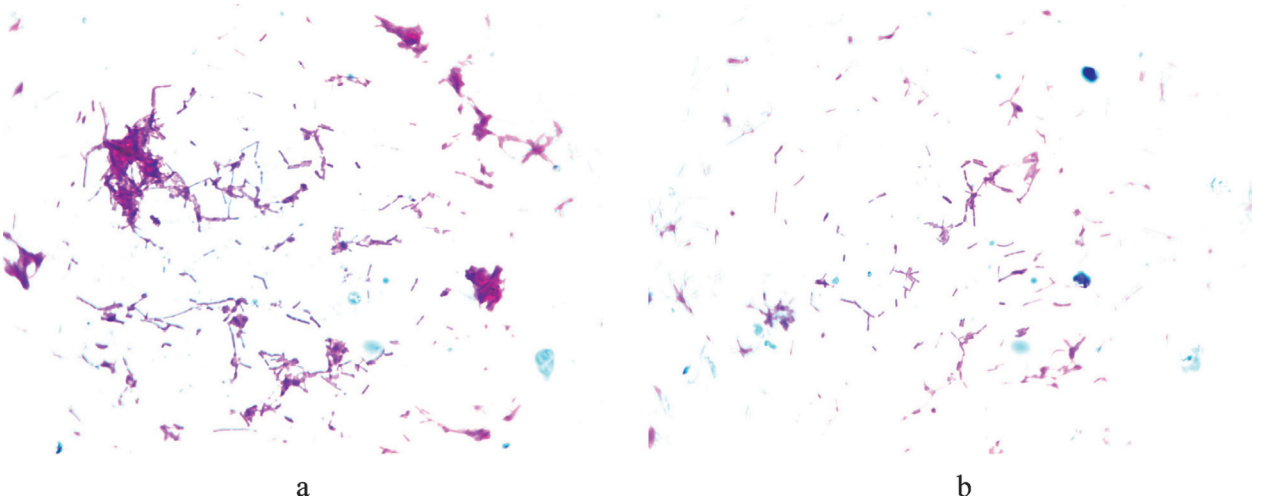

Figure 6. Optical microscope photograph of untreated (a) and microwave radiated (b) *E. coli* cells.

DISCUSSION

It has been determined that rapid inactivation of each examined bacterial cells were observed in the first 60 sec. After this period no considerable change was observed on the amount of viable cell and it fixed as the optimum contact time (Fig 1). The results demonstrate that the maximum destruction level occurred in a short time. The rapid inactivation process is a significant parameter for large scale application in industrial request. Similar fast bacterial inactivation trend (110 sec) by microwave effect on *Staphylococcus aureus* cells was reported in a previous study and this finding was attributed to heat transfer from the stainless steel substrate and a little direct energy was observed from the microwaves [12].

The reports by some researchers suggest that microwave energy absorption by biological materials can be measured by temperature increase of the sampling material [1, 13]. In order to evaluate effect of temperature for the reducing quantity of live cells of bacteria in microwave, temperature changes were monitored with a thermometer. As can be seen from the Fig. 2 changes in the temperature of bacterial cell suspension was found relative to microwave exposure time. The viable counts in each cell suspensions were found to significantly reduce relative to an increase in the microwave heating temperatures. Similar results has been also reported by the other researchers for effect of microwave on *E. coli* [3, 12].

When the exposure time at 60 sec and 360 power of microwave were kept constant, initial bacterial cells were changed from 0.5 to 2 in accordance with MacFarland standarts. As indicated in Fig. 3, the effect of microwave on the bacterial cells decreased with increasing of the amount of bacterial cells as an expected. But, the maximum microwave effect on the cell viability were observed from *E. coli* cells at the same conditions

The experiments were carried out using various power of microwave under the determined optimum exposure time and the initial bacterial cell quantity. The maximum effect was obtained from full power as an expected for the each examined bacteria. This indicates particular suitability power of 900 for the reducing viable cells of examined bacteria.

According to Woo *et al.*, 2000, general indication of heat injure to microorganisms is the leakage of nucleic acid and protein from the cells. Microwave damaged cells have also been reported to release purines and pirimidines in a solution [14]. It is well known that, nucleic acids and its related compounds are absorb UV light at 260 nm. Also, the presence of these materials and intracellular proteins in a solution indicates harm to the cell at the membrane level [3]. For that reason, the existence of nucleic acid released into the cell suspension was analysed by measuring the absorbance at 260 nm (Fig 5). *E.coli* and *B. cereus* were showed that similar patterns in their release of nucleic acid. But, *S. aureus* was presented more resistant to microwave than others examined bacteria in this respect.

Figure 6 represent the optical microscope images of *E.coli* cells taken at 1000 magnification by an light microscope (Olympus). Figure 6a corresponds to the reference (untreated) while Fig. 6b corresponds to the microwave treated cells. As can be seen in Fig 6 there are a difference in the shape of remaining of the bacteria. The reduce of bacterial burden may explaning of destruction by microwave irradiation of the cell wall structure. These images indicates that the microwave treatment reducing amount of *E.coli* cells.

In order to find out the effect of water activity on the microwave efficiency on the viability of bacterial cells various amount of sterile glycerol added into sterile saline solution containing bacterial cells. The water activity was measured with water activity equipment (Aqualab). Water activity was changed from 0,994 to 0,752. With decrease in the water activity the effect of microwave on the examined bacteria was not changed under determined optimum conditions. We think that, water activity may be is an important parameter for the microwave using sterilization techniques in food and related industry. Moreover, Vela and Wu (1979) reported that microorganisms were inactivated only when in the presence of water and that dry or lyophilized organisms were not affected by microwave. Therefore, microwave sterilization is not suitable for dry food.

The results obtained from these experiments, using continuous power application of 2450 MHz microwaves at the different power levels, indicated that the microwaves apparently produced lethal effects on the examined bacteria by heat generated during microwave exposure.

REFERENCES

[1] Vela GR, Wu JF. 1979. Mechanism of lethal action of 2,450-MHz radiation on microorganisms. Applied and Environmental Microbiology. 37:550-553.

[2] Rosenberg U, Bogl W. 1987. Microwave thawing, drying and baking in the food industry. Food Technology 41:85-91.

[3] Woo IM, Rhee IK, Park HD. 2000. Differential damage in bacterial cells by microwave radiation on the basis of cell wall structure. Applied and Environmental Microbiology. 66:2243-2247.

[4] Fujikawa H, Ushioda H, Kudo Y. 1992. Kinetics of Escherichia coli destruction by microwave irradiation. Applied and Environmental Microbiology. 58:920-924.

[5] Godblith SA, Wang DIC. 1967. Effect of microwaves on Escherichia coli and Bacillus subtilis. Applied Microbiology. 15:1271-1375.

[6] Lechowich RV, Beuchat LR, Fox KI, Webster FH. 1969. Procedure for Evaluating the Effects of 2,450-Megahertz Microwaves upon Streptococcus faecalis and Saccharomyces cerevisiae. Applied Microbiology. 17:106-110.

[7] Heddleson RA, Doores S, Anantheswaran RC. 1994. Parameters affecting destruction of Salmonella spp. by microwave heating. Journal of Food Science. 59:447–451.

[8] Welt BA, Tong CH, Rossen JL, Lund DD. 1994. Effect of microwave radiation on inactivation of Clostridium sporogenes (PA 3679) spore. Applied and Environmental Microbiology. 60:482-488.

[9] Shin JK, Pyun YR. 1997. Inactivation of Lactobacillus plantarum by pulsed-microwave irradiation. Journal of Food Science. 62:163-166.

[10] Farber JM, Aoust JYD, Diotte M, Sewell A, Daley E. 1998. Survival of Listeria spp. On raw whole chickens in microwave ovens. Journal of Food Protection. 61:1465-1469

[11] Olsen CM. 1965. Microwaves inhibit bread mold. Food Engineering. 37:51-54.

[12] Yeo CBA, Watson IA, Stewart-Tull DES, Koh VHH. 1999. Heat transfer analysis of Staphylococcus aureus on stainless steel with microwave radiation. Journal of Applied Microbiology. 87:396-401

[13] McRee DI. 1974. Determination of the absorption of microwave radiation by a biological specimen in a 2450 MHz microwave field. Health Phys. 26:385-390.

[14] Khalil H, Villota R. 1988. Comparative study on injury and recovery of Staphylococcus aureus using microwave and conventional heating. Journal of Food Protection. 51:181-186.