

การยับยั้งเชื้อจุลินทรีย์อย่างรวดเร็วโดยรังสีไมโครเวฟ

Fast Disinfection of Microbial Cultures Using Microwave Radiation

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บทคัดย่อ

ไมโครเวฟเป็นคลื่นแม่เหล็กไฟฟ้าไม่มีประจุมีความถี่ระหว่าง 0.3 and 300 กิกะเฮิรตซ์ การศึกษาเป็นการทดสอบประสิทธิภาพการทำลายเชื้อจุลินทรีย์ที่ก่อให้เกิดโรคด้วยผลของไมโครเวฟ รังสีไมโครเวฟในสารละลายแบคทีเรียมีผลทำให้ลดจำนวนการมีชีวิตเนื่องจากมีการเพิ่มขึ้นของอุณหภูมิในสารละลาย ไมโครเวฟทำให้เซลล์ได้รับความร้อนและทำลายเซลล์แบคทีเรียส่วนใหญ่ ไมโครเวฟทำลายเซลล์แบคทีเรียทำให้เซลล์แตกสลายได้ง่าย ในการศึกษาสารละลายแบคทีเรียได้รับไมโครเวฟ ได้ทำการศึกษาเปรียบเทียบอัตราการยับยั้งขึ้นกับเวลาและพลังงานของไมโครเวฟ ได้เปรียบเทียบผลการทดลองโดยจำนวนโคโลนีต่อมิลลิลิตรเพื่อสรุปนัยสำคัญ ความแตกต่างระหว่างจำนวนโคโลนีต่อมิลลิลิตรของจำนวนแบคทีเรียที่เท่ากันที่ได้รับรังสีไมโครเวฟ ประสิทธิภาพสูงสุดของไมโครเวฟเกิดขึ้นที่ 3 นาทีของการได้รับรังสี ผลการศึกษาแสดงว่าไมโครเวฟมีผลการยับยั้งต่อเชื้อแบคทีเรียโดยทำให้เกิดความร้อนระหว่างการได้รับรังสีไมโครเวฟ ได้ศึกษาผลของความร้อนและพลังงานแม่เหล็กไฟฟ้าของไมโครเวฟต่อเซลล์แบคทีเรีย ผลการศึกษาแสดงว่าความร้อนจากไมโครเวฟมีผลต่อการมีชีวิตของแบคทีเรียและการทำลายการซึมผ่านของเยื่อเซลล์ ผลการศึกษานี้แสดงว่าแม่เหล็กไฟฟ้าจากไมโครเวฟมีผลต่อแบคทีเรีย การจัดตั้งป็นเบื้อน และการนำกลับมาใช้ใหม่ของน้ำที่ได้รับการบำบัดแล้วจะมีผลต่อการลดต้นทุน เวลา งบประมาณ และแรงงานต่อระบบอุตสาหกรรมและเป็นผลต่อการรักษาสภาวะแวดล้อมให้ดีขึ้น

คำสำคัญ: ไมโครเวฟ การยับยั้ง ไมโครเวฟ

Abstract

Microwaves are non-ionizing electromagnetic waves with frequencies between 0.3 and 300 GHz. The study was tested for their efficiency in destroying the pathogenic bacteria with effect of microwave radiation. Microwave radiation in bacterial cell suspensions resulted in a dramatic reduction of the viable counts according to the increase of the final temperature of the cell suspensions. The microwave-heated cells revealed severe damage of most bacterial cells. Microwave-injured bacterial cells were easily lysed. In this study, suspension of bacterial cells were exposed to microwave. The degrees of inactivation by the various time and different power of microwave were compared systematically. When each result was compared with

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the CFU/ml results obtained bacterial suspensions, significant conclusions were attained. The difference between the CFU/ml values of similar bacterial suspensions subjected to microwave radiation. Maximum efficiency of microwave was observed at 3 min exposure time. The results show that microwaves apparently produced inactivation effects on the examined bacteria by heat generated during microwave exposure. Thermal and electromagnetic (specific) effects in responses of bacterial cells to high-power microwave were studied. It was demonstrated that microwave heating cells resulted in a loss of viability and damage of permeability of membranes. These results illustrate the existence of an electromagnetic action of a microwave field to the bacterial cell organisms. The removal of contaminants and reuse of the treated water would provide significant reductions in cost, time, liabilities, and labour to the industry and result in improved environmental resource management.

Keywords: microwave, disinfection, bacteria

Introduction

The use of microwave irradiation in the cooking and processing of foods has become remarkably popular in recent years. There have been several publications in recent years which have mentioned possible lethal effect of microwave on microbial species especially bacteria.¹⁻³ Nowadays, also, the use of microwave radiation has become popular in the industrial applications particularly food and related industry.^{2,4}

Although many investigators have studied the destruction of microorganisms by microwave irradiation,^{5,6} the mechanism of destruction has not been fully clarified.³ It is thought that the destruction of microorganisms might be due mainly to induction heating by microwave exposure.^{1,7}

In recent years, the use of microwave radiation has become popular in the food industry for thawing, drying, and baking foods, as well as for the inactivation of microorganisms in foods.^{4,8} In particular, microbial destruction by

microwave radiation has great potential in the pasteurization of foods.⁴ Its short heating and exposure time is less destructive to food than longer conventional heating.³ All of these works have led to the conclusion that microwave radiation extends food preservation by reducing microbial cells in food.

Water is an essential nutrient for all known forms of life and the mechanisms by which fluid and electrolyte homeostasis is maintained in humans are well understood.⁹ Microbiologically contaminated drinking water is a cause of community-acquired infection. Contaminated water is estimated to result in illness more than half a million per year.¹⁰

Water treated with microwave should be another way to sanitary for drinking water. This study investigated the microbial cell inactivation in water by microwave heating along with the differences in the effects on gram-positive and gram-negative bacteria including *E. coli*, *B. subtilis*, *S. typhimurium*, *S. sonnei*, *S. aureus*.



Materials and Methods

Bacterial strains

The bacterial strains used were *E. coli*, *B. subtilis*, *S. typhimurium*, *S. sonnei*, *S. aureus*. All are cultured on nutrient agar slants.

Preparation of bacterial suspensions

Each bacterium in suitable broth medium was cultured overnight at 37°C with shaking. The bacterial culture was transferred to a sterilized test tube, and centrifuged at 4,000 rpm for 10 min. Cells were harvested by centrifugation and washed twice with a sterile 0.9% NaCl solution. The cell pellets were resuspended in a 0.9% NaCl solution at a cell concentration of 10^9 to 10^{10} CFU/ml, which was used for the microwave radiation.

Microwave treatment of water

Two hundred milliliter water filled in a 500 ml screw capped glass bottle was given continuous microwave (2450 MHz) exposure at 200-700 W a microwave oven (Electrolux EM30EC90SS). Cap of the bottle was kept slightly loose so that steam generated during heating can get released. Baseplate (30 cm diameter) of the oven rotated at a speed of 5 rpm.

Enumeration of viable cells

After microwave irradiation, the bacterial suspension was transferred from the tube into broth, and rinsed. An aliquot of the broth was serially diluted with saline, and viable cells were enumerated by colony counting. The remainder of the broth was incubated at 37°C without further treatment.

Results and Discussions

Figure 1 shows the trend of temperature rising course at various power levels. As indicated the higher the microwave power output level was, the higher the temperature of water could reach. Heating rate was occurred when microwave power level was set at 200 W or higher. It could be observed that at power of 700 W initial rapid increases in temperature were eventually followed by a slow down temperature increase finally reach a constant.

Temperature measured around 100°C within 6, 4 and 3 min under microwave radiation at power levels of 200, 500 and 700 W, respectively. In this study, the thermal treatment experiment was followed by medium power level of microwave at 500 W.

When water in a screw capped glass bottle was irradiated at 25°C, the temperature increased linearly up to 80°C, according to the exposure time (Figure 1). From the slope of the plotted line, the rate of temperature increase was calculated as 0.30, 0.37 and 0.57°C/s at 200, 500, and 700 W, respectively.

The raw data points shown in Figure 1 and Figure 2 illustrate typical survival curves of each tested bacteria at 500 W.

The effect of power was investigated in the range of 200, 500, and full power 700 W and the results were presented in Figure 2.

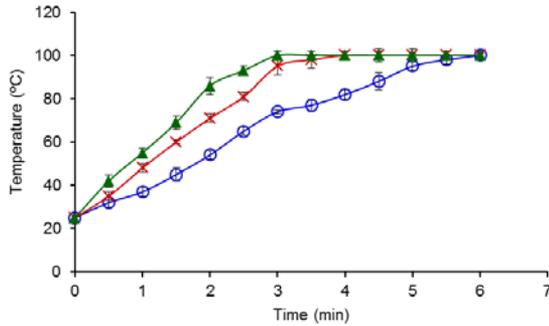


Figure 1 Increase in temperature of water caused by microwave exposure. Water was irradiated at 200 (○), 500 (×), and 700 (▲) W.

Figure 2 shows the typical nonlinear concave upwards semilogarithmic inactivation curves as influenced by microwave power. These results indicate that the slope increased with increasing microwave power and that lower microwave power had a less pronounced effect. Similar tendencies were presented by for others bacteria at different microwave power, where at higher microwave power, the steepness of the curve was more pronounced effect to inactivate bacteria.

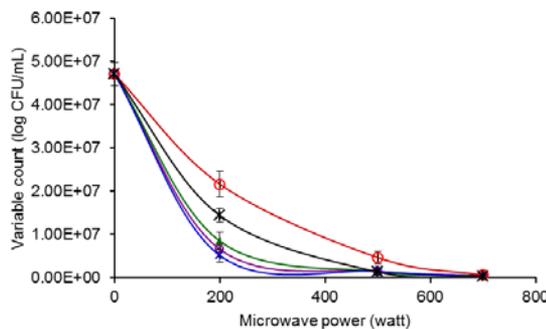


Figure 2 Changes in viable bacterial cells counts caused by microwave irradiation for 3 min. Bacteria suspension was exposed at 200-700 W. Symbols: ×, *S. sonnei*; ▲, *S. typhimurium*; ◆, *E. coli*; *, *B. subtilis*; ○, *S. aureus*.

Figure 3 was shown that changes in bacterial cells concentrations with microwave

exposure time presenting the typical sigmoidal semilogarithmic inactivation curves as influenced by microwave at 500 W.

In general, the log counts of bacteria gradually decreased with microwave exposure time under conditions microwave 500 W. The rate of inactivation increased with microwave exposure time. The thermal inactivation curves, however, were not always sigmoidal.

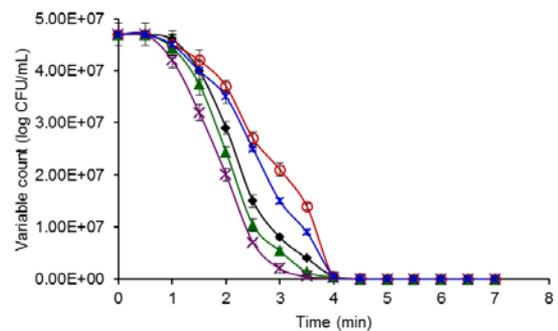


Figure 3 Representative survival curves of each examined bacteria suspended in water. Symbols: ×, *S. sonnei*; ▲, *S. typhimurium*; ◆, *E. coli*; *, *B. subtilis*; ○, *S. aureus*.

The survival curve of *S. aureus* was apparently the most nonlinear among all the survival curves. The bacterial concentration did not seem to change significantly at the early stage of the process. As the microwave exposure time exceeded a certain threshold, which varied with bacteria, the rate of bacterial inactivation began to accelerate. The first period of microwave exposure time (about 1-1.5 min, where the variable bacterial cell count did not decrease, was observed in Figure 1. This effect observed in a microwave inactivation study can be caused by the dimension of the samples (Juneja et al., 2006). If a sample used in a microwave inactivation is thick or contained in a large container, the temperature at the geometric center of the sample cannot be increased to the desired



temperature immediately. Time is required for heat to diffuse into the geometric center of the sample. During this initial period, the bacteria localized at the geometric center are not significantly affected by the microwave exposure time. At lower microwave power, the sampling times were significantly longer than the first 1 min period of microwave exposure time (data not shown). This effect observed in this study may be characteristic of certain biological factors where a thermal microwave process must higher than initial energy barrier before a lethal effect can be observed.¹¹

As shown in Figure 1 changes in the temperature of water were related to microwave exposure time and in Figure 2 showed relationship between viable bacterial cells and microwave power. The viable bacterial cells reduced relatively to an increase in the microwave heating temperatures and microwave power. It has been determined that inactivation of each examined bacterial cells were observed after the 1 min. After 4 min no considerable change was observed on the amount of viable cell of *S. sonnei*, *S. typhimurium* and 6 min for *E. coli*, *B. subtilis* and *S. aureus* (Figure 3). The results demonstrate that the destruction level occurred in 3-6 min. This rapid inactivation process could be a significant factor for large scale application in industry.

It was reported that microwave energy could be absorbed by biological molecules that could be measured by increasing of temperature.^{1,12} In order to evaluate effect of temperature for the reducing quantity of variable bacterial cells in microwave, temperature changes were monitored with a thermometer.

Figure 3 shows the effects of microwave exposure time on the bacterial cells. The results showed the decrease of bacterial cells with increasing of microwave exposure time. The maximum microwave effect on the cell viability was observed from *S. sonnei* at the same conditions. The experiments were performed using 200-700 W of microwave power. The results in Figure 3 showed microwave 500 W was high enough for total inactivation of viable cells of examined bacteria. Microwave 700 W was able to inactivate total viable bacterial cells in the shorter time (3 min) while microwave 200 W could also inactivate the viable bacterial cells but in longer time (8 min) (data not shown). The reduction of viable cells due to microwave damage bacterial cells by heat destruction of cell wall structure and causing leakage of nucleic acid and proteins from the cell into solution.²

Conclusions

The results showed the examined gram negative bacteria were less resistant to microwave than gram positive bacteria. This study was performed by continuous microwave power application of 2450 MHz with different power. The results suggested that microwaves produce lethal effects of bacteria by heat generation.

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