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Note

Kinetic Analysis of Microbial Inactivation by Microwave Irradiation based on Temperature History

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The kinetics of microbial inactivation by microwave irradiation were studied from the viewpoint of thermal inactivation analysis. From the temperature history of a microbial suspension during irradiation, a thermal inactivation curve was predicted. The predicted curves were similar to experimentally produced curves by irradiation at various energy outputs, showing that the inactivation was predominantly due to the heat induced by irradiation. On the other hand, a small difference in the inactivation rate between the curves was observed at a high output. This implied the possible existence of nonthermal factors in irradiation lethality.

Key words : Microwave irradiation/Thermal inactivation/Microbial inactivation kinetics/Nonthermal lethality.

Many investigators have studied the inactivation of microorganisms by microwave irradiation (Brown and Morrison, 1954; Cunningham, 1980; Fruin and Guthertz, 1982). The inactivation was considered to be mainly due to induction heating by irradiation (Fung and Cunningham, 1980; Vela and Wu, 1979), but the mechanism of microbial inactivation has not been fully clarified (Fung and Cunningham, 1980). Also, little is known about the inactivation kinetics of microorganisms as affected by irradiation. Recently Fujikawa et al. found that the inactivation kinetics of several species of bacteria (vegetative cells) as affected by microwave irradiation at 2,450 MHz can be approximated by a series of three first-order inactivations (Fujikawa et al., 1992; Fujikawa and Ohta, 1994). Further, it was found that there was no remarkable difference in the microbial inactivation patterns produced by microwave irradiation and those by conventional heating under conditions in which treatments had given rise to similar temperature histories for a microbial suspension (Fujikawa et al., 1992). The result suggested that microbial inactivation during irradiation would be predominantly due to its thermal effect.

There are several studies that have shown the existence of nonthermal effects of microwave irradiation on microbial inactivation and metabolic activity (Culkin and Fung, 1975; Dreyfuss and Chipley, 1980; Olsen, 1965; Wayland et al., 1977; Web and Dodds, 1968). Kozempel et al. (1998) described four predominant theories for the nonthermal effects including selective heating, electroporation, cell membrane rupture, and magnetic field coupling. On the other hand, many reports have denied the existence of nonthermal lethal effects of irradiation (Coote et al., 1991; Goldblith and Wang, 1967; Mudgett, 1982; Welt et al., 1994). Therefore it is well worth studying the inactivation kinetics produced by microwave irradiation from the viewpoint of thermal inactivation analysis, for evaluation of the thermal effect of microwave irradiation on microbial inactivation. However, this approach has not yet been utilized. In this study we predicted the thermal inactivation curves of *Escherichia coli* from the temperature histories during microwave irradiation. Then we compared these inactivation curves with those which were experimentally produced.

The materials and methods for experiments were the same as those of our previous study (Fujikawa et al., 1992). An *E. coli* suspension was irradiated at

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100, 200, and 300 W.

The thermal inactivation of the microorganism was considered to follow a first-order reaction in equation (1).

$$\ln (N/N_0) = -\int k(t)\,dt \qquad (1)$$

where N and N_0 are the numbers of viable cells per unit volume at treatment times t and 0, respectively, and k is an inactivation rate constant. For the temperature dependency of $k(t)$ in equation (1), the Arrhenius equation in equation (2) (Arrhenius, 1889) was introduced.

$$k = A\exp(-E_a/RT) \qquad (2)$$

where A is a frequency factor, E_a is the activation energy of inactivation, R is the gas constant, and T is the absolute temperature and a function of time. Substitution of the right hand term of equation (2) for k in equation (1) yielded equation (3).

$$\ln(N/N_0) = -\int A\exp(-E_a/RT)\,dt \qquad (3)$$

Equation (3) represents the survivor ratio, N/N_0 at time t in any type of temperature history.

The temperature, T, of the *E. coli* suspension during irradiation increased linearly at a rate that corresponded to the microwave energy output. Thus, during irradiation, T was expressed in equation (4).

$$T = a + bt \qquad (4)$$

where a and b are the initial temperature and the rate of temperature increase, respectively. The value of a was 20℃ for all experiments. Values of b were 0.2, 0.29, and 0.50℃/s at 100, 200, and 300 W, respectively. The survivor ratio during irradiation was calculated with equation (3). The equation was numerically solved with the trapezoidal rule.

Thermal inactivation curves predicted from the temperature histories at various energy outputs were quite similar to the experimentally produced curves (Fig. 1). These results showed that inactivation of the microorganism during irradiation was predominantly due to the heat induced by the irradiation. Especially, at a low output of 100W the predicted curve was identical to the experimental one. This also suggested that the temperature non-uniformity in the cell suspension during irradiation did not lead to any remarkable difference in microbial inactivation kinetics in this study.

There were small differences in the predicted and experimentally generated inactivation kinetics at high outputs. First, the experimental curve showed a drop a little earlier than the predicted one (Figs. 1 b and 1 c). Second, the predicted inactivation curves were curvilinear with upward convexes, whereas the experimental curves could be approximated with a series of three linear portions; it was more remarkable at a higher energy output, e. g., 300 W (Fig. 1 c). The reasons for these differences in kinetics are not understood at present. The differences, especially the

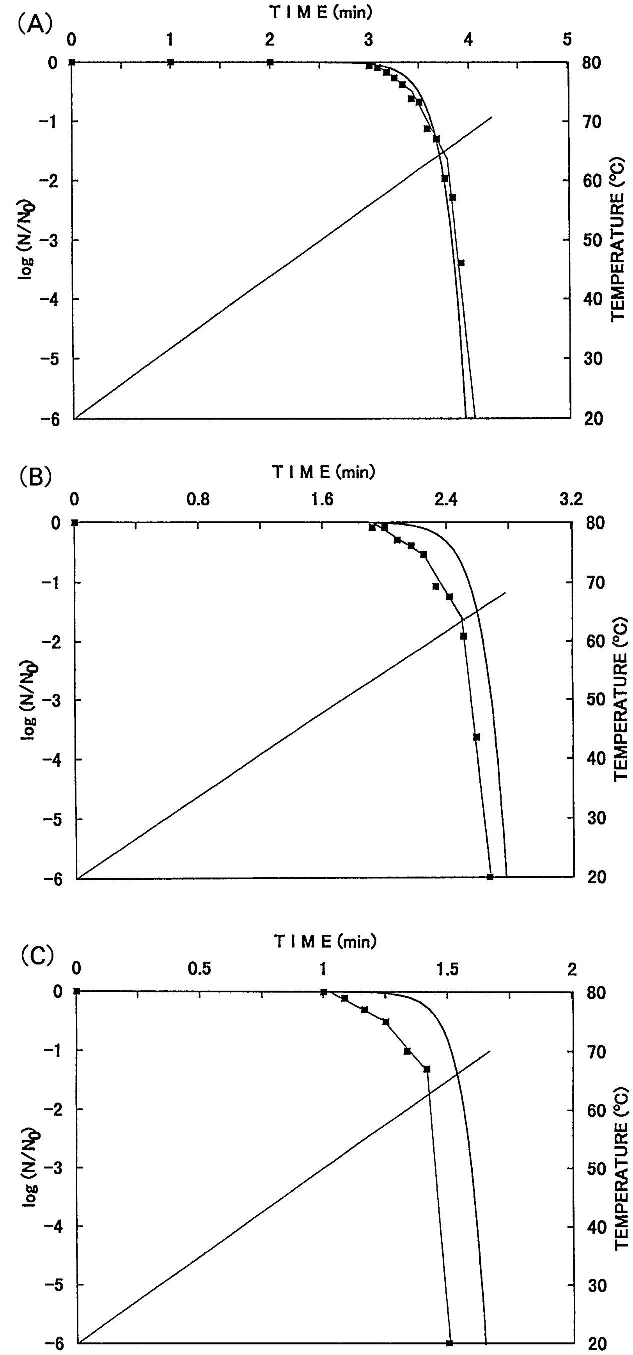

FIG. 1. Thermal inactivation analysis of *E. coli* by microwave irradiation at 100 w (A), 200 W (B), and 300 W (C). Experimentally produced lines are those connecting experimental data points. Predicted lines are smooth curves.

former, might be due to a nonthermal lethal factor involved in microwave irradiation.

Recently Welt et al. (1994) reported that nonthermal lethal effects of microwave irradiation were not found. However, the energy output that they used for their study was rather low, i.e., 80 and 150 W. In our study, at a lower energy output the difference between the experimental and predicted inactivation curves was small (Fig. 1a). Also, in our previous study, there were no significant differences in the microbial inactivation curves produced by microwave irradiation with low energy output (100 W) and those produced by conventional heating at b=0.076 ℃/s (Fujikawa et al. 1992). On the other hand, Kozempel et al. (1998) reported on the microbial inactivation with microwave irradiation at reduced temperatures below 40℃. In their study, as much as 5,000−5,400 W of microwave energy was applied. However, regardless of the tremendously high energy used, the magnitude of microbial cell reduction by microwave irradiation was small and was less than 1 log unit reduction in most cases (Kozempel et al. 1998). Therefore, our study and those reported in the literature suggested that the nonthermal lethal effects of microwave irradiation, if any, would be very small compared with the thermal effect of irradiation and that the nonthermal lethality could be observed at a high energy output.

On the other hand, Tsuchido et al. (1982) reported that a preheating at a linearly rising temperature (b =0.01 ℃/s) gave *E.coli* cells thermotolerance at 50 ℃. Thus, physiological changes caused by heat in microbial cells in the process of thermal inactivation might not be negligible when analyzing microbial inactivation kinetics during microwave irradiation.

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