EFFECT OF FAR-INFRARED IRRADIATION ON PASTEURIZATION OF BACTERIA SUSPENDED IN LIQUID MEDIUM BELOW LETHAL TEMPERATURE

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The purpose of this study is to examine the influence of far-infrared irradiation on pasteurization of bacteria suspended in liquid medium below the lethal temperature. Under this condition, *Escherichia coli* and *staphylococcus aureus* are injured and killed by far-infrared irradiation. With increase in irradiation power and with decrease in depth of the suspension, the ratio of the number of injured cells to the number of viable cells becomes higher, and the number of viable cells becomes smaller. Moreover, the pasteurization effect can be enhanced by raising the bulk temperature of the suspension. By estimating the temperature distribution within the suspension, it is suggested that the test bacteria are injured and killed in the very thin domain near the surface of the suspension.

Introduction

The technology for producing far-infrared heaters made of fine ceramics with high emissivity in the infrared region of wavelength longer than 3 μm and low emissivity in the infrared region of wavelength shorter than 3 μm has improved, and such far-infrared heaters are now manufactured in Japan. Far-infrared radiation (FIR) is widely utilized in our daily life despite the fact that few fundamental studies on its applications have appeared.

In the food industry, the systems for processing food materials to food products are increasing and are growing more complicated. So, it is expected that new technology for preventing microbial pollution of foodstuffs will be established.

Organic materials and water, which are the main components of foodstuffs and microorganisms, absorb considerable FIR energy, and especially radiative energy in the wavelength bands near 3 and 6 μm. Moreover, FIR is safe for foodstuffs and human beings. Furthermore, since no heating medium is needed in the case of far-infrared radiative heating, by applying FIR to thermal operations such as pasteurization, many merits may be expected which will improve the cleanliness of working environments and lead to direct pasteurization of wrapped foodstuffs, easy operation (thermal control), simplification of apparatus, etc. Therefore, the application of FIR to pasteurization in food processing is desirable, and fundamental studies of its application are needed.

Shimada[15] and Van Zuilichem *et al.*[17] reported only the possibility of pasteurization by far-infrared irradiation. The interaction of the vibration mode between organism and FIR is proposed[3]. However, non-thermal effects of FIR on organisms are poorly understood[5]. Fundamental studies on the application of FIR to pasteurization based on experimental results are thus required.

Previously, we examined experimentally the influence of FIR on pasteurization of *Escherichia coli* and *Staphylococcus aureus* suspended in phosphate-buffered saline (PBS, pH 7.0)[7]. In that work, the pasteurization effect of far-infrared irradiation (radiative heating) was compared with that of thermal conductive heating (a conventional method) under the condition that the transient behavior of the bulk temperature of the suspension irradiated by FIR was the same as that heated by thermal conduction. The number of viable cells in the suspension irradiated by FIR was much less than that heated by thermal condition, and the ratio of the number of sublethally injured cells[12,16] (shortened to "injured cells" hereafter) to the number of viable cells was remarkably higher than that heated by thermal conduction. Moreover, it was suggested that the pasteurization effect was due both to the absorption of radiative energy by the bacterial suspension in a very thin domain near the surface and to the bulk temperature

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of the bacterial suspension.

To apply FIR to pasteurization in food processing, it is necessary to understand more exactly why far-infrared irradiation was superior to thermal conductive heating for pasteurization of bacteria suspended in PBS. The present study aims at examining the influence of far-infrared irradiation on pasteurization of bacteria suspended in PBS below the lethal temperature. We also examine whether bacteria in a suspension kept below the lethal temperature are killed by far-infrared irradiation or not, and discuss the mechanism from the viewpoint of radiative heat transfer.

1. Experimental Apparatus and Methods

1.1 Preparation of sample

Test organisms *E. coli* 745 and *S. aureus* 9779 stored at the Tokyo Metropolitan Research Laboratory of Public Health were used. The test organisms were cultured in Brain Heart Infusion broth (Difco) at 308 K for 24 h on a reciprocal shaker.

Sample The culture was suspended in 0.05M-PBS (pH 7.0) to give a final bacterial concentration of about 10^9 CFU·dm^{-3} (CFU: Colony-Forming Unit). The bacterial suspension was dispensed in a petri dish made of stainless steel ($14.5 \times 88.5\,\text{mm}\phi$).

The side wall of the petri dish was thermally insulated, and the insulator was covered with aluminium foil to reflect infrared radiation. The petri dish with the bacterial suspension was placed on a plate, which was controlled at a specified temperature in the region from 263 to 283 K by using a coolant. The cooling plate was on a reciprocal shaker (**Fig. 1**).

1.2 Far-infrared irradiation

Irradiation apparatus The irradiation chamber (W0.43 × D0.32 × H0.58 m) is made of aluminium plates, and a far-infrared heater made of a mullite cylinder ($300 \times 15\,\text{mm}\phi$) with a reflector is placed at the top. The same irradiation apparatus was used in our previous study[7].

Irradiation Irradiation distance was 0.15 m. The surface temperature of the heater was from 773 to 943 K.

A specified rate of electric power was supplied to the heater. After the surface temperature of the heater and the room temperature reached a steady-state condition, the petri dish with the suspension was placed under the heater and irradiation was carried out. During irradiation, the suspension was being stirred at 180 rpm. After a specified time passed, the petri dish was put on a plate kept at 278 K and the suspension was cooled rapidly.

1.3 Microbial counts

The cooled suspension was diluted with sterile saline. The diluent was pour-plated with the same agar medium as used in our previous study[7]. For *E. coli*, Nutrient Agar (Eiken Chemicals) was used as the non-selective medium[7], and Nutrient Agar plus sodium deoxycolate (Wako Chemicals) was used as the selective medium[7]. For *S. aureus*, Standard Method Agar (Eiken Chemicals) was used as the non-selective medium, and Standard Method Agar plus sodium chloride (Wako Chemicals) was used as the selective medium. The colonies were enumerated after incubation at 308 K for 48 h.

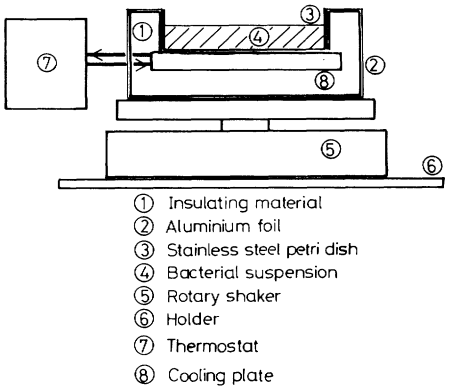

Fig. 1. Sample for pasteurization by far-infrared irradiation

1.4 Measurement of suspension temperature

CA-thermocouples sheathed by a stainless steel tube ($0.25\,\text{mm}\phi$) were placed horizontally at a position close to the surface, at the center and at the bottom of the suspension. The temperatures at these three points in the suspension were recorded during irradiation.

1.5 Measurement of irradiation power

Irradiation power was estimated experimentally from the evaporation rate of the water layer irradiated by infrared radiation. The methods were the same as those used in the previous study[9].

2. Experimental Results

2.1 Effect of irradiation power

To determine the influence of far-infrared irradiation on pasteurization of bacteria suspended in PBS, FIR pasteurization was performed under the condition that the pasteurization effect by thermal conductive heating was negligible.

Figure 2 shows the effect of irradiation power, q_{ir}, on the transient behavior of the bulk temperature of the bacterial suspension irradiated with the heater and cooled from the bottom. The bulk temperatures are lower than 313 K, and the pasteurization effect by thermal conductive heating are negligible.

The pasteurization results under the condition shown in Fig. 2 are indicated in **Fig. 3**, which shows the effect of q_{ir} on the ratio of the colony-forming unit of the test bacteria irradiated by FIR for 20 min to that of the control (N_l/N_{l0}). For *E. coli*, the decrease of survivors grown on the non-selective agar

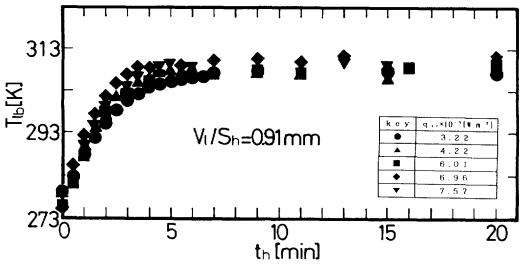

Fig. 2. Effect of irradiation power on transient behavior of bulk temperature of bacterial suspension

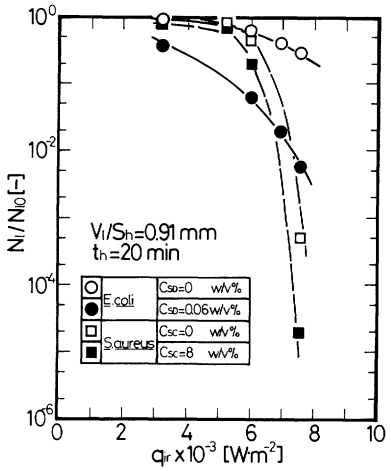

Fig. 3. Effect of irradiation power on N_l/N_{l0} of *E. coli* and *S. aureus*

(C_{SD}=0 w/v%) is slight. However, in the case of the selective agar (C_{SD}=0.06 w/v%), N_l/N_{l0} decreases with increase in q_{ir}.

For *S. aureus*, in the cases of both the non-selective agar (C_{SC}=0 w/v%) and the selective agar (C_{SC}=8 w/v%), the decrease of N_l/N_{l0} is slight in the region of q_{ir} lower than about 7×10^3 W·m^{-2}, but N_l/N_{l0} decreases markedly in the region of higher q_{ir}. As shown in Fig. 3, the test bacteria are injured and killed by far-infrared irradiation under the condition that the pasteurization effect by thermal conductive heating was negligible.

To examine the effect of q_{ir} more exactly, we examined the influence of the concentration of the selective reagent on the colony-forming unit. For magnitude of injury, it can be considered as follows. We define the cells that are able to form colonies on the non-selective agar, but not on the selective agar at lower concentration of the selective reagent, as type I. And we define the cells that are able to form colonies on both the non-selective agar and the selective agar at lower concentration, but not on the selective agar at higher concentration, as type II. The cells of type I are injured more seriously than those of type II.

Figure 4 shows the effect of the concentration of the selective reagent in the agar medium, C_{SD}, on

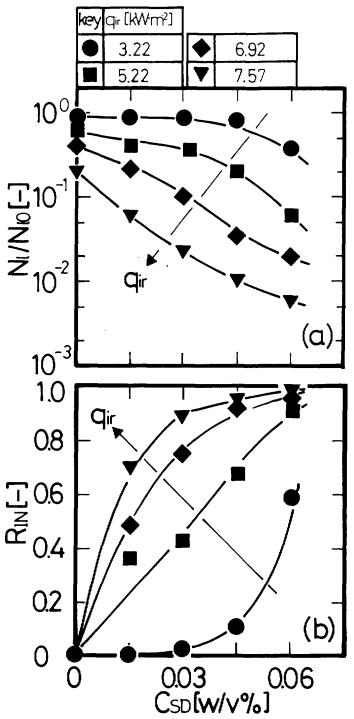

Fig. 4. N_l/N_{l0} and R_{IN} of *E. coli* vs. C_{SD}

N_l/N_{l0} and R_{IN}[7] of *E. coli*. R_{IN} is the ratio of the number of injured cells to the number of survivors and is calculated by the following equation.

$$R_{IN}=\frac{N_l(C=0)-N_l(C=C)}{N_l(C=0)}=1-\frac{N_l(C=C)}{N_l(C=0)} \quad (1)$$

With increase in q_{ir}, N_l/N_{l0} is lower and R_{IN} is higher at any concentration of the selective reagent. Moreover, with increase in q_{ir}, the fraction of more seriously injured cells (type I) is becoming higher and that of more lightly injured cells (type II) is becoming lower.

2.2 Effects of depth and of bulk temperature of bacterial suspension

In our previous study[7] it was suggested that the pasteurization effect was due to the absorption of radiative energy by the bacterial suspension in a very thin domain near the surface and due to the bulk temperature of the bacterial suspension. So, under the condition that the bulk of the bacterial suspension was kept below the lethal temperature, the effect of depth of the suspension on the pasteurization by far-infrared irradiation was examined.

Figures 5 and 6 show the effects. The abscissa, V_l/S_h, is the ratio of the volume of the suspension to the area irradiated by FIR, and is equivalent to the depth of the suspension. For both *E. coli* and *S. aureus*, N_l/N_{l0} decreases with decrease in V_l/S_h, and the differences between N_l/N_{l0} on the non-selective agar and N_l/N_{l0} on the selective agar become larger. In short, R_{IN} becomes higher. And the decrease of

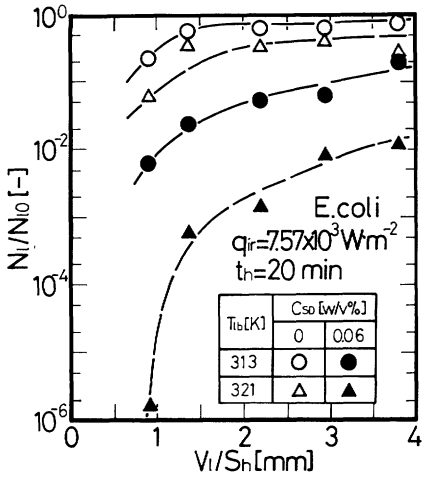

Fig. 5. Effect of depth and bulk temperature of bacterial suspension on N_l/N_{l0} of *E. coli*

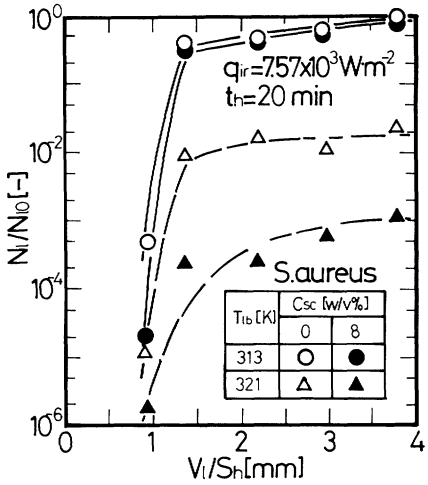

Fig. 6. Effect of depth and bulk temperature of bacterial suspension on N_l/N_{l0} of *S. aureus*

N_l/N_{l0} for *S. aureus* is more remarkable than that for *E. coli*.

Furthermore, when the bulk of the suspension was kept below the lethal temperature of the test bacteria, we performed pasteurization by far-infrared irradiation at the bulk temperatures of 313 K and 321 K in order to examine the effect of the bulk temperature of the suspension on pasteurization. The effects on the pasteurization are shown in Figs. 5 and 6. N_l/N_{l0} in the case of $T_{lb}=321$ K decreases more markedly than that in the case of $T_{lb}=313$ K, and the difference between the colony-forming number on the non-selective agar and that on the selective agar become larger as the bulk temperature of the suspension becomes high, from 313 K to 321 K. Thus the pasteurization effect by far-infrared irradiation can be enhanced by raising the bulk temperature of the suspension, even under the lethal temperature.

3. Discussion

By estimating the temperature distribution in a very thin domain near the surface of the suspension irradiated by FIR, we discuss the experimental results of pasteurization by far-infrared irradiation from the viewpoint of radiative heat transfer.

3.1 Estimation of surface temperature of bacterial suspension

It is assumed that the optical and thermal properties of the bacterial suspension are equal to those of water. As the suspension is being stirred during irradiation, the bulk temperature is kept constant. We will consider the heat transfer in a suspension lump close to the surface of the suspension, which is irradiated by FIR and heated. As it is thinkable that the radiative heat transfer rate is very high by comparison with the agitation rate of the suspension, the lump temperature may be assumed to rise before the lump goes to the bulk of the suspension. So we focus our attention on the time behavior of the lump temperature while the lump is close to the surface of the suspension.

The lump is heated from the upper side by the FIR heater used in the pasteurization experiments. Within the lump, heat transfer is assumed to be one-dimensional. As it is thinkable that the initial temperature equals the bulk temperature, we consider the unsteady-state temperature distribution within the lump. Therefore, the temperature distribution within the lump is to satisfy the following differential equation with the initial condition and the boundary conditions.

$$\frac{\partial}{\partial x}\left[\kappa_w\frac{\partial T_l}{\partial x}-\varepsilon_w q_{ir}\exp(-a_w x)\right]=C_{pw}\rho_w\frac{\partial T_l}{\partial t_h} \quad (2)$$

I.C. : $t_h=0,\ 0\leqq x\leqq V_l/S_h;\ T_l=T_{lb}$ (3)

B.C. 1: $t_h>0,\ x=V_l/S_h;\ T_l=T_{lb}$ (4)

B.C. 2: $t_h>0,\ x=0;\ \kappa_w\left(-\frac{\partial T_l}{\partial x}\right)+h_G(T_l-T_a)$

$$+\varepsilon_w\sigma(T_l^4-T_a^4)+k_G\rho_{as}\left(\frac{Y_s^*}{1+Y_s^*}-\frac{Y_a}{1+Y_a}\right)\gamma_w=0 \quad (5)$$

In Eq. (2), it is assumed that the temperature of the lump is so much lower than the surface temperature of the heater that emission within the lump is negligible. The first term of the left-hand side of Eq. (5) is the conductive heat flux[11] within the lump, the second term is the convective heat flux[1,2,4,10,11] on the lump, the third term is the radiative heat flux[13] from the surface of the lump, and the fourth term is latent heat flux[2,6,10,11,14] due to evaporation. x is the depth from the surface of the lump. α_w is

the avarage absorption coefficient of water over the wavelength in the infrared region, and is obtained by the following equaion.

$$\alpha_w = \frac{\int_0^\infty \alpha_{w\lambda}\varepsilon_{h\lambda}I_{B\lambda}d\lambda}{\int_0^\infty \varepsilon_{h\lambda}I_{B\lambda}d\lambda} \quad (6)$$

$\alpha_{w\lambda}$ is the monochromatic absorption coefficient of water[8], and $\varepsilon_{h\lambda}$ is the monochromatic emissivity of the heater[7]. $I_{B\lambda}$ is calculated by using Planck's equation at the surface temperature of the heater. In the calculation of α_w, Eq. (6) is integrated numerically over the wavelength, λ, from 0.75 μm to 20 μm.

3.2 FIR pasteurization mechanism

Figure 7 shows the calculation results of the temperature distribution close to the surface of the suspension, and indicates the effect of irradiation time on the temperature distribution. The estimated surface temperature of the lump rises to 326.2 K for a very short irradiation time of 0.1 s, which almost equals the lethal temperature. The surface temperature rises to 353.4 K for an irradiation time of 0.3 s and over 373 K for an irradiation time of 0.5 s. As the bacterial suspension is agitated at 180 rpm, the time of an agitation cycle is about 0.33 s. However, in the present experiments it is very difficult to connect the irradiation time with the time of the agitation cycle. So, taking $t_h = 0.3$ s as a measure of irradiation time, we consider the temperature distribution within the lump.

Figure 8 shows the calculation results of the temperature distribution, and indicates the effect of irradiation power on the temperature distribution. In the case of $q_{ir} = 7.57 \times 10^3\ \mathrm{W \cdot m^{-2}}$, the surface temperature of the lump rises to 353.4 K, and the domain of temperature higher than the lethal temperature expands with increase in q_{ir}. Moreover, with increase in q_{ir}, N_l/N_{l0} is lower (Fig. 3), and the fraction of more seriously injured cells becomes higher and that of more lightly injured cells becomes lower (Fig. 4(b)). If inactivation of the test bacteria is due to the pasteurization by thermal conductive heating, N_l/N_{l0} of both *E. coli* and *S. aureus* are estimated to be less than 10^{-8} at 353.4 K for 0.3 s by using the following equations.

$$(N_l/N_{l0})_{calc} = \exp\left(-\int_0^{t_h} k dt_h\right) \quad (7)$$

$$k = A \cdot \exp(-E_a/RT_{lb}) \quad (8)$$

In the calculation of Eq. (8), literature values[7] of the frequency factor, A, and the activiation energy, E_a, are used.

Figures 9 and **10** show the calculation results of the temperature distribution close to the surface of the suspension, and respectively indicate the effect of the dimensionless depth of the suspension, $x/(V_l/S_h)$, and

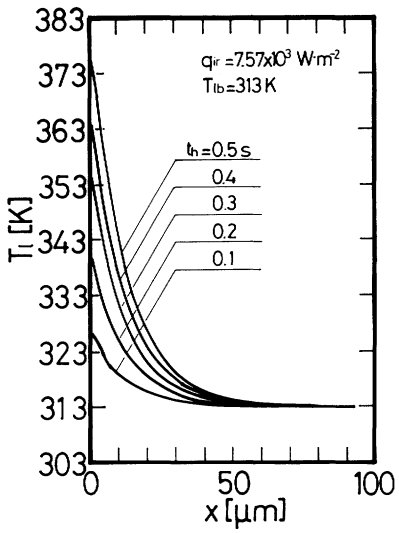

Fig. 7. Effect of irradiation time on temperature in a very thin domain near surface of water layer

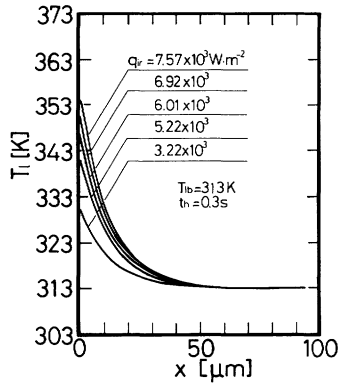

Fig. 8. Effect of irradiation power on temperature in a very thin domain near surface of water layer

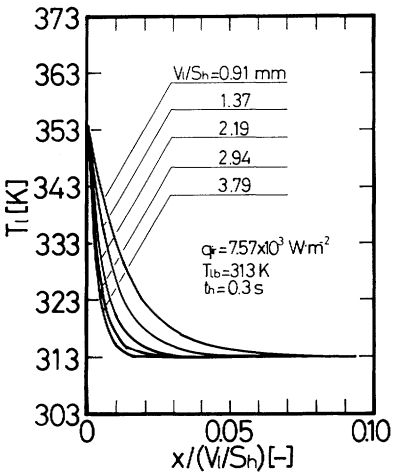

Fig. 9. Effect of depth of water layer on temperature in a very thin domain near surface

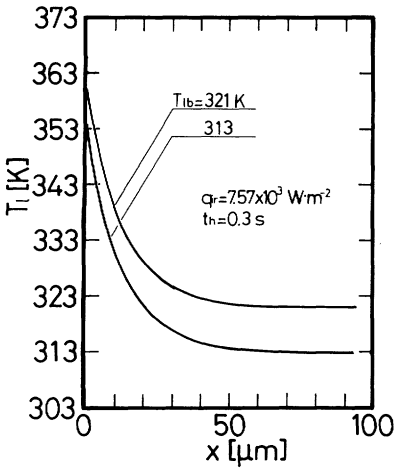

Fig.10. Effect of bulk temperature of water layer on that in a very thin domain near surface

the effect of the bulk temperature of the suspension, T_{lb}, on the temperature distribution. As shown in Fig. 9, the domain of $x/(V_l/S_h)$ of higher temperature than the lethal temperature of the test bacteria expands with decrease in V_l/S_h. As shown in Fig. 10, with increase in T_{lb} the surface temperature of the lump becomes high, and the domain of higher temperature than the lethal temperature expands markedly.

With the relationship between the pasteurization results and the temperature distribution close to the surface of the suspension estimated by using Eqs. (2), (3), (4) and (5), the mechanism of pasteurization by far-infrared irradiation of the test bacteria suspended in PBS below the lethal temperature may be predicted as follows. As the suspension is being stirred during irradiation, lumps in the suspension appear near the surface and disappear successively. The test bacteria are stressed in the very thin domain near the surface of the suspension, whose temperature is very high. Therefore, one after another the test bacteria in the domain near the surface of the suspension return to the bulk. This circulation is repeated. Then, the test bacteria stressed successively in the domain may be injured and killed. However, to get a grasp of the details of the pasteurization effect by far-infrared irradiation, the pasteurization effect by thermal conductive heating under the condition that the suspension reciprocates successively from high temperature to low temperature in a very short interval must be determined.

Concluding Remarks

Pasteurization by far-infrared irradiation of *E. coli* and *S. aureus* suspended in PBS was performed under the condition that the suspension was kept below the lethal temperature. The influences of three factors (irradiation power, suspension depth and suspension bulk temperature) on pasteurization are studied experimentally and from the viewpoint of radiative heat transfer. The following results and conclusions are obtained.

1) The test bacteria are injured and killed by far-infrared irradiation under the condition that the bulk of the suspension is kept below the lethal temperature.

2) With increase in irradiation power and with decrease in depth of the suspension, the ratio of the number of injured cells to the number of viable cells becomes higher, and the number of viable cells becomes smaller. Moreover, the pasteurization effect by far-infrared irradiation can be enhanced by the bulk temperature of the suspension.

3) By estimating the temperature distribution near the surface of the suspension, it is suggested that the test bacteria are injured and killed in the very thin domain near the surface.

Nomenclature

A	=	frequency factor defined by Eq. (4)	[s^{-1}]
C	=	concentration of selective reagent in selective medium	[w/v%]
C_p	=	heat capacity	[$J \cdot kg^{-1} \cdot K^{-1}$]
E_a	=	activation energy obtained from Eq. (4)	[$J \cdot mol^{-1}$]
h_G	=	heat transfer coefficient for natural convection	[$W \cdot m^{-2} \cdot K^{-1}$]
I_B	=	radiative intensity of black body	[$W \cdot m^{-2}$]
k	=	chemical reaction rate constant for pasteurization obtained from Eq. (4)	[s^{-1}]
k_G	=	mass transfer coefficient for natural convection	[$m \cdot s^{-1}$]
N_l	=	concentration of viable cells in bacterial suspension	[$CFU \cdot dm^{-3}$]
q_{ir}	=	irradiation power	[$W \cdot m^{-2}$]
R	=	gas constant (=8.314)	[$J \cdot K^{-1} \cdot mol^{-1}$]
R_{IN}	=	defined by Eq. (1)	[—]
S_h	=	area of bacterial suspension irradiated by infrared radiation	[m^2]
t_h	=	heating time	[s]
T_l	=	temperature of bacterial suspension	[K]
V_l	=	volume of bacterial suspension	[dm^3]
x	=	depth of water layer	[m]
Y	=	absolute humidity	[kg-H_2O/kg-dry air]
α	=	absorption coefficient	[m^{-1}]
γ	=	latent heat of vaporization	[$J \cdot kg^{-1}$]
ε	=	emissivity	[—]
κ	=	thermal conductivity	[$W \cdot m^{-1} \cdot K^{-1}$]
λ	=	wave length	[μm]
ρ	=	density	[$kg \cdot m^{-3}$]
σ	=	Stefan-Boltzmann's constant (=5.67×10^{-8})	[$W \cdot m^{-2} \cdot K^{-4}$]

〈Subscripts〉

a	=	air
b	=	bulk
h	=	infrared heater
s	=	surface
SC	=	sodium chloride
SD	=	sodium deoxycolate
w	=	water

λ = monochromatic
0 = initial state

〈Superscript〉
* = saturated

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