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Death Rate Constants of *Escherichia coli* by Heated Dolomite Powder Slurry

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Bactericidal action of dolomite powders heated at 600-1000℃ against *Escherichia coli* was kinetically investigated. Dolomite powder heated at 650℃ or higher exhibited bactericidal action. The *E. coli* death in the heated dolomite powder slurries followed first-order reaction kinetics, the death rate constant (k) was determined. The value of k increased with increasing dolomite powder concentration, and the dilution coefficient (n), which shows the dependence of k on the concentration of reagent, was measured. The n values of the powders heated at up to 750℃ and at over 800℃ were almost identical to those of MgO and CaO, respectively. This suggests that the first emergence of bactericidal action at approximately 700℃ is corresponding to generation of MgO, and the second emergence at over 800℃ is due to generation of CaO. The slurry temperature was found to significantly affect the bactericidal action. The slope of the Arrhenius plot of k for *E. coli* grown at 37℃ exhibited a discontinuous point at approximately 22℃, at which activation energy for the death of *E. coli* in the heated dolomite powder slurry changed. This temperature corresponds to that of the phase transition of cell membrane lipids.

Key words: dolomite, antibacterial activity, bactericidal action, magnesium oxide, calcium oxide

Introduction

Dolomite includes $CaCO_3$ and $MgCO_3$ as the main components. Because dolomite can be orally taken, part of a dolomite powder has been used as a food additive to supply minerals. The authors have previously studied the effects of ceramic powders on the bacterial growth. Twenty-six kinds of ceramic powders, such as metallic oxide and carbide, were examined for their antibacterial activity, and about ten kinds of powders were found to inhibit bacterial growth (1). CaO and MgO, in particular, exhibited strong antibacterial activity (2, 3). Through heat treatment, the main components of dolomite, $CaCO_3$ and $MgCO_3$, are converted to CaO and MgO, respectively, which exhibits antibacterial activity (4, 5).

Recently, microbial pollution and degradation caused by microorganisms has created serious problems in various industrial fields, especially food processing (6-8). The use of the heated dolomite in food processing is therefore not only expected to be a source of minerals but also to prolong the shelf life of foodstuffs. Moreover, CaO and MgO were not mutagenic, but rather, reduced the mutagenicity of mutagens, such as benzo[a]pyrene, 2-nitrofluorene and methylglyoxal (9, 10). Because of the development of antimicrobial agents that are very safe for human and the natural environment is desired, this heated dolomite will have many applications as an antibacterial agent in a wide range of fields.

In the previous study (11), the antibacterial activity was examined using the conductance method capable of measuring the electrical conductivity change caused when bacteria metabolizes and produce more mobile charged molecules from large molecules, and minimal inhibitory concentration was measured. However, there are no studies on the kinetic analysis of antibacterial activity of the heated dolomite. In the present study, the bactericidal action of dolomite powders heated at 600-1000℃ against *Escherichia coli* was kinetically investigated.

Materials and Methods

Test bacteria

Escherichia coli 745, which were obtained from the Tokyo Metropolitan Research Laboratory for Public Health. The bacterium was stored at −80℃ and thawed and incubated in Brain Heart Infusion broth (Eiken Chemicals, Tokyo, Japan) at 37℃ for 20 h. The culture was then suspended in sterile physiological saline (0.85 w/v%) at approximately 10^8 colony forming units (cfu)/ml. The bacterial suspension was kept in ice water before being used in the experiments.

Preparation of sample

The composition of dolomite (Kawatetsu Miming Co. Ltd., Gifu, Japan) produced in Gifu prefecture is shown in **Table 1**. The dolomite powder was heated between 600 and 1000℃ in air for 1 h and ground in a planetary ball mill. The mean particle size of the heated powder was approximately 5 μm. A powder slurry was prepared by suspending the ground dolomite powder in saline.

Table 1 Composition of dolomite powder.

	CaO	MgO	SiO_2	Fe_2O_3	Water
(%)	31.1	18.5	20.9	0.16	<0.5

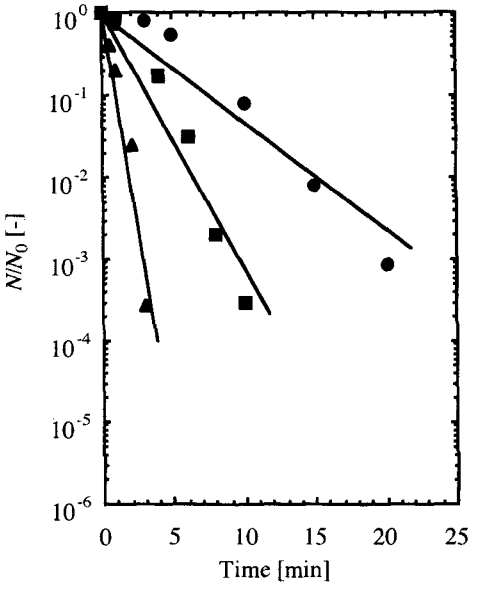

Fig. 1 Effect of bactericidal action of dolomite powder heated at 1000℃ against *E. coli.* ●; 0.05 mg/ml, ■; 0.1 mg/ml, ▲; 0.25 mg/ml.

Viable counts

Powder slurry (20 ml) was poured into a vial (internal diameter 35 mm) and agitated using a magnetic stirrer at 250 rpm. The slurry temperature was controlled using a water bath. The bacterial suspension was pipetted into the slurry. The initial bacterial concentration was approximately 10^6 cfu/ml. From time to time, a sample (100 μl) was withdrawn and diluted in saline. The diluted samples were pour-plated with Nutrient Agar (Eiken Chemicals, Tokyo, Japan). Duplicate plates were used for each dilution. The colonies were counted after incubation at 37℃ for 48 h.

Results and Discussion

Effect of powder concentration

Figure 1 demonstrates the *E. coli* death in the slurry of dolomite powder heated at 1000℃. The slurry temperature was 37℃. The heated dolomite powder exhibited bactericidal action on *E. coli.* At a powder concentration of 0.25 mg/ml, over three-orders of magnitude reduction of the survival ratio was observed within 3 min. An increase in powder concentration enhanced the bactericidal action. When the logarithmic survival ratio and treatment time were employed as the ordinate and abscissa, respectively, the population of *E. coli* was seen to decrease almost linearly. Thus, assuming that the death of *E. coli* by the heated dolomite powder follows first-order kinetics expressed by Eq. (1), the

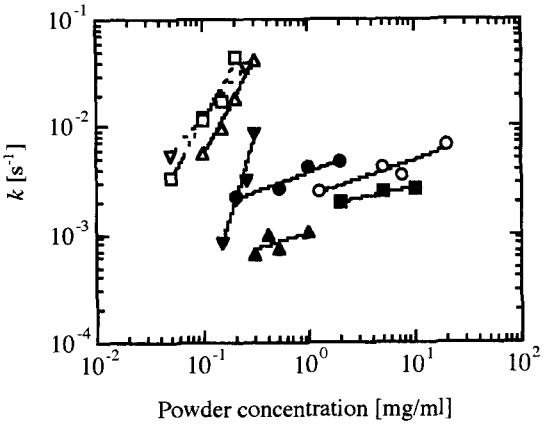

Fig. 2 Death rate constants of dolomite powder heated at different temperatures for *E. coli.*○; MgO, ▲; 650℃, ■; 700℃, ●; 750℃, ▼; 800℃, △; 900℃, ▽; 1000℃, □; CaO.

first-order death rate constant (k) can be determined.

$$dN/dt = -kN \quad (1)$$

Figure 2 shows the death rate constant. The k values increased with increasing powder concentration. Also, the following relationship between k and reagent concentration (C) is well known (12):

$$k = \alpha C^n \quad (2)$$

where α and n are an empirical constant and the dilution coefficient, respectively. n represents the dependence of k on reagent concentration. The value of n was obtained from the slope of the line in Fig. 2, and the dolomite powder heated at 1000℃ was $n = 1.2$.

Effect of heating temperature

The bactericidal effect of heated dolomite is considered to be due to CaO and/or MgO, which are produced through heat treatment. Thus, the relationship between k and the heating temperature was investigated. Okouchi *et al.* (4) reported that, over 650℃, thermal decomposition of dolomite is :

$$MgCa(CO_3)_2 \rightarrow MgO + CaCO_3 + CO_2 \quad (3)$$

and over 900℃ :

$$MgCa(CO_3)_2 \rightarrow MgO + CaO + 2CO_2 \quad (4)$$

Fig. 2 summarizes the effect of heating temperature on k values. Dolomite powder heated at 650℃ or higher exhibited bactericidal action. The powder heated at 600℃ did not exhibit the bactericidal action even at 10 mg/ml for 1 h (data not shown). The n values of the powder heated at lower 750℃ and at over 800℃ were almost identical to those of MgO and CaO, respectively. This suggests that the first emergence of bactericidal action is corresponding to generation of MgO, and the second emergence at over 800 ℃ is due to generation of CaO. The k values of the powder

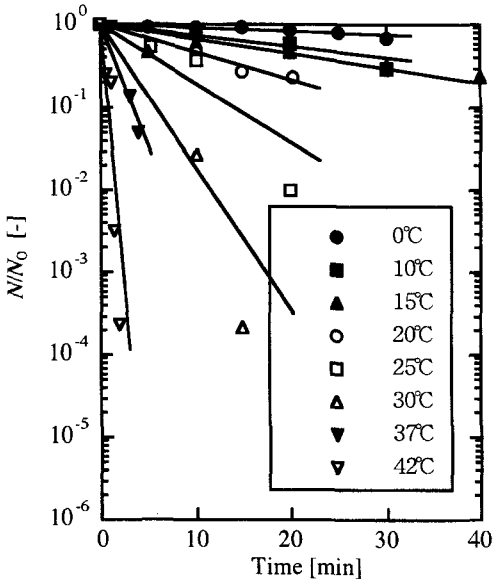

Fig. 3 Effect of slurry temperature of bactericidal action of dolomite powder heated at 1000℃ against *E. coli*.

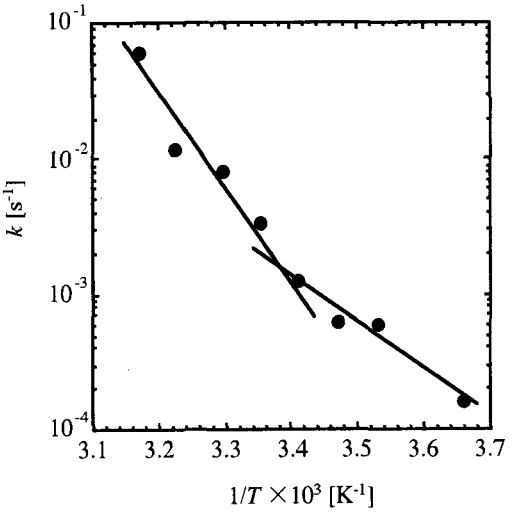

Fig. 4 Arrhenius plot of death rate constant of *E. coli* treated by dolomite powder heated at 1000℃.

Table 2 Activation energy required *E. coli* death by dolomite powder heated at 1000℃.

	Range	E_a [J/mol]
①	Heated dolomite	1.35×10^5
②	Heated dolomite	2.26×10^4
①	CaO	1.48×10^5
②	CaO	4.13×10^4

heated at over 900℃ were comparable to those of pure CaO. Considering Ca content shown in Table 1, the bactericidal action of heated dolomite powder is greater than that of pure CaO. Okouchi *et al.* (4) described that this may be due to high activity of surface of the heated dolomite powders.

Both MgO and CaO, which are the main components, have high pH value in the slurry sate, approximately 10 and 12, respectively. This high alkalinity is considered to be a main factor of the antibacterial activity. Sugiyama *et al.* (13) observed the concentrated OH^- layers formed on the surface of these materials by infra-red spectrophotometer (FT-IR) measurement. And, they described that the bactericidal action was considered to depend on a synergistic effect of both the direct contact of bacterial cells with the concentrated OH^- layers and the contact of OH^- ions diffused from the surface into the bulk solution (13). However, in our previous study (14-16), injuries to bacteria caused by the CaO and MgO powder slurries were studied on the basis of a change in sensitivity to antibiotics. Although the pHs of the both slurries are high, the changes in antibiotic sensitivities caused by these slurries were obviously different from those caused by alkaline treatment. Mendonca *et al.* (17) described that high pH- treatment did not damage cell, growing equally well on both selective and non-selective media. In addition, they proposed that the effects of high-pH were all or nothing event. Our results agree well with their results (14-16). Therefore, the CaO and MgO powder slurries obviously has an antibacterial factor separated from the alkaline effect. For the CaO and MgO, the generation of active oxygen such as superoxide anion was observed from the powder slurry (18). The sensitivity changes in response to the CaO and MgO were in agreement with those induced by active oxygen (15, 16). The bactericidal action of these powders, apart from the alkaline effect, may originate from the active oxygen species.

Effect of slurry temperature

The effect of slurry temperature on k of the heated dolomite for *E. coli* was examined within a temperature range over which there is no reduction in the viability of *E. coli* cells. The dolomite powder heated at 1000 ℃ was used, and the slurry concentration was 0.2 mg/ml. The temperature significantly affected the bactericidal action of the dolomite powder slurry on *E. coli* (**Fig. 3**). At 42℃, a five-orders of magnitude reduction in the survival ratio was observed within 2 min.

Figure 4 shows the relationship between the values of k and slurry temperature (Arrhenius plot). The slope of the Arrhenius plot changed noticeably at approximately 22℃. The k values of the dolomite powder heated at 1000℃ were almost equal to those of the CaO (see Fig. 3). The CaO and MgO also exhibited the discontinuous point in the slope at approximately 22 ℃ (19, 20). The values of activation energy (E_a) were obtained from the following equation (**Table 2**):

$$k = A \exp(-E_a/(RT)) \quad (5)$$

where, A, R and T are the frequency factor, gas constant and reaction temperature, respectively. E_a for the dolomite powder markedly changed at 22℃

and was almost equal to that of CaO (Table 2).

In *E. coli*, the membrane functions such as substrate transport, activities of the membrane-associated enzymes, and the maintenance of cell integrity, depend on membrane fluidity. It seems likely that the temperature of growth and the heating temperature influence membrane fluidity. When the temperature of the cells is above a critical level, a gel-liquid crystalline phase transition of the membrane phospholipids should occur (21). Sinensky (22) showed that the temperature of the phase transition of membrane lipids in *E. coli* was usually at 21 to 23℃ for cells grown at 37℃. The discontinuous point in Fig. 4 is approximately equal to this temperature of phase transition. At the phase transition temperature, the cell membrane undergoes a change in phase from a highly-ordered gel to a disordered liquid crystalline state (23). Therefore, membrane fluidity may affect the bactericidal action of the dolomite powder. The phase transition of the cell membrane induces a change in conformation of charged molecules, such as proteins on the surfaces of cells, which causes a variation in the interaction between the cells and the powder. Also, the permeability of the membrane might vary due to changes in membrane fluidity.

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