Transactions of the Materials Research Society of Japan 25 [4] 1075-1078 (2000)

Apparent Death Rate Constants of *Escherichia coli* in CaO Powder Slurry

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The death process of *Escherichia coli* in the CaO powder slurry followed a first-order reaction kinetics. The values of the apparent death rate constants (k) increased with an increase in powder concentration and were larger than those of NaOH solution with the same pH as the CaO powder slurry. The slurry temperature significantly affected the bactericidal action of the CaO powder slurry on *E. coli*. The slope of the Arrhenius plot of k changed at the slurry temperature of approximately 22°C. It is probable that this change in activation energy required for the death of *E. coli* in CaO powder slurry is due to the phase transition of the cell membrane.

Key words: antibacterial activity, bactericidal action, calcium oxide, *Escherichia coli*, death rate constant

1. INTRODUCTION

Many goods and materials finished with antimicrobial reagents have been manufactured, and inorganic antimicrobial materials have recently been used for the prevention of biodeterioration and biodegradation in many fields[1, 2]. Most of them use ceramic materials as their supports and immobilize antimicrobial metals, such as silver and copper, on their surfaces[3, 4]. However, there is currently a great interest in the use of a ceramic that itself has antimicrobial activity[5-7], and a fundamental study on the antimicrobial activity of these ceramics is needed.

It was reported that calcium oxide (CaO) exhibited a strong antibacterial activity. The CaO powder slurries acted against both gram-positive and gram-negative bacteria in a bactericidal manner [8]. This powder slurry showed an efficacy against the spores of *Bacillus subtilis* which have high resistivities to heat and antibacterial reagents [9]. Also, the CaO did not show a mutagenicity, on the contrary, it completely reduced the mutagenicity of 2-nitrofluorene [10, 11]. Thus, application of these metallic oxides to foodstuffs and environmental applications is expected.

However, there are no studies concerning the kinetic analysis of the antibacterial activity of the CaO powders. In this study, we have determined the death rate constants the CaO powder slurry on *Escherichia coli*. In addition, the effect of temperature on the bactericidal action of the CaO powder was investigated.

2. EXPERIMENTALS

2.1 Test bacteria

Escherichia coli 745 was obtained from the Tokyo Metropolitan Research Laboratory for Public Health. The bacteria were cultured in Brain Heart Infusion (BHI) broth (Eiken Chemicals Co. Ltd.) at 37°C for 20 h with a reciprocal shaker. The culture was then suspended in sterile physiological saline to yield a final bacterial concentration of approximately 10^8 colony forming units (CFU)/ml.

2.2 Determination of death rate constants

Calcium oxide (CaO: Kishida Chemicals. Co. Ltd.) was used as the test material. The mean particle size of the CaO powder was 2.7 μm. The powders were heated at 180°C for 20 min and suspended in sterilized saline to

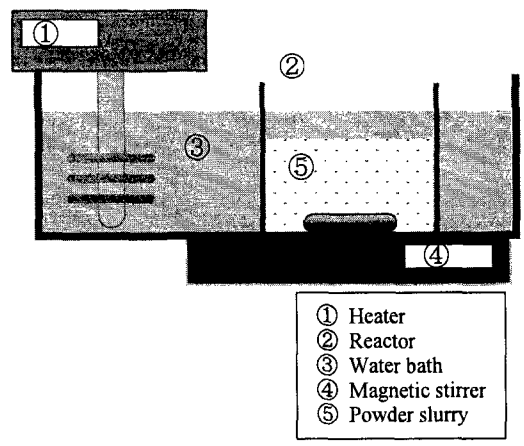

Fig. 1 Schematic drawing of apparatus

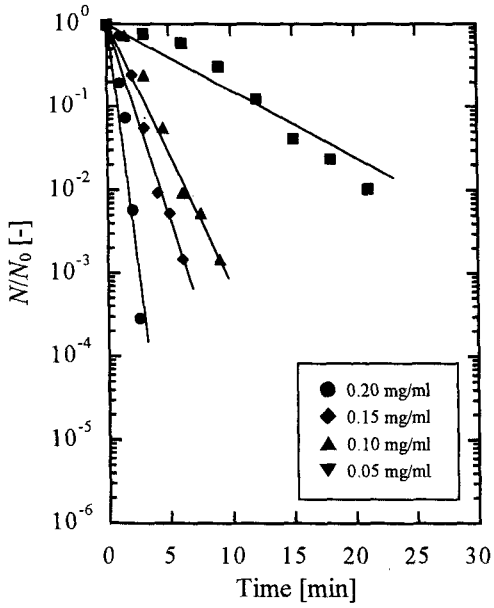

Fig. 2 Bactericidal action of slurry concentration of CaO

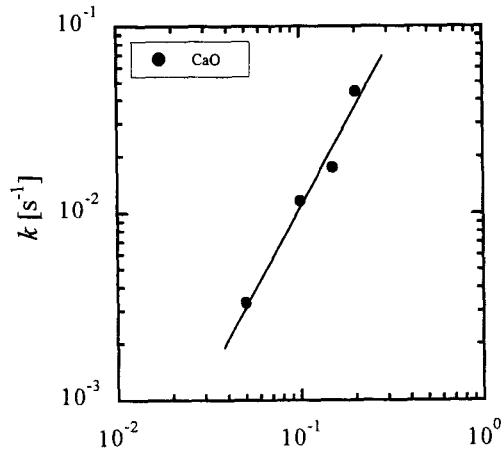

Fig. 3 Relationship between death rate constant and slurry concentration of CaO powder

yield the specified concentration. **Figure 1** illustrates the schematic drawing of the apparatus. A 20 ml aliquot of the powder slurry was poured into a vial with an inner diameter of 32 mm and agitated with a magnetic stirrer at 250 rpm. The experimental temperature of the slurry was controlled using a water bath. A 0.2 bacterial suspension was pipetted into the slurry. From time to time, a sample was withdrawn and diluted with saline. The diluted samples were pour-plated with Nutrient Agar (Eiken Chemicals). Duplicate plates were used for each dilution. The colonies were enumerated after incubation at 37°C for 48 h.

3. RESULTS & DISCUSSION

3.1 Effect of powder concentration

Figure 2 shows the bactericidal action of the CaO powder slurry against *E. coli* at 37°C. The ordinate, N/N_0, is the survival ratio of *E. coli*. The initial concentration of the viable cells of *E. coli* (N_0) was approximately 10^6 CFU/ml. An increase in the concentration of the CaO powder slurry enhanced the bactericidal action on *E. coli*. And, as shown in Fig. 2, the logarithmic survival ratio of *E. coli* decreased linearly with time. Hence, assuming that the death of *E. coli* by the CaO powder follows a first-order reaction kinetics as shown in Eq. (1), the first-order death rate constant (k) was determined.

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

Figure 3 shows the results. The values of k increased with increasing concentration of the CaO powder slurry. The dilution coefficient (n), which represents the dependence of k on the CaO powder concentration, is described by the following equation.

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

where α is an empirical constant. As shown in Fig. 3, the CaO powder slurry had a dilution coefficient of 1.77 for *E. coli* at 37°C.

Because the CaO powder slurry has a high pH values, the bactericidal action of the powder slurry was compared with that of alkaline solution ($NaOH_{aq}$). As shown in **Fig. 4**, the values of k of the CaO powder slurry were larger than those of NaOH solution at the same pH. This suggests that the CaO powder slurry has a greater bactericidal action than the alkaline effect. Sugiyama *et al.* [12] synthesized a silicate-containing hydroxyapatite (SiAp) using CaO, Na_2HPO_4 and SiO_2. The SiAp slurry also had a high alkali at a pH of approximately 11.0. Also, its bactericidal action was considered to be caused by a synergistic effect of both the direct contact of bacterial cells with the concentrated OH^- layers formed on the surface of the suspended SiAp particles and the contact of OH^- ions diffused from the surface of SiAp into the bulk solution. However, in our previous study[13], injuries to bacteria caused by the CaO powder slurry were studied on the basis of a change in sensitivities to antibiotics. As the result, though the CaO powder slurry has high pH value, the changes in the antibiotic sensitivities by the CaO powder slurry were obviously different from those by alkaline treatment. The CaO powder slurry would have the other antibacterial factors as well as the alkaline effect. For the CaO, the generation of an active oxygen, such as superoxide anion, was

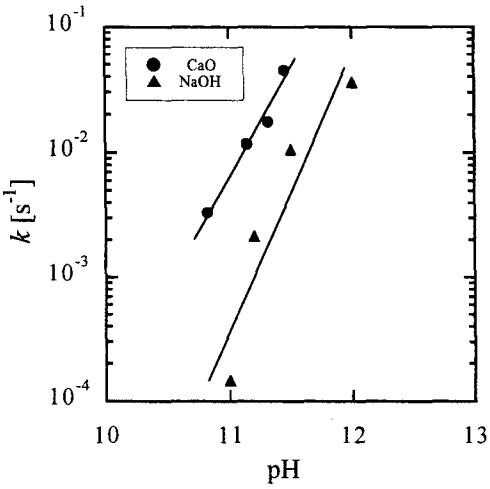

Fig. 4 Comparison of bactericidal action between CaO powder and NaOH solution

observed from the powder slurry[14]. Active oxygen species are very reactive and powerful oxidizing agents. They are known to injure and kill the bacteria[15, 16]. In fact, the changes in antibiotic sensitivities by the active oxygen treatment were similar to those by the CaO powder slurry[17]. Therefore, it was considered that the active oxygen generated from the powder is one of the primary factor in the antibacterial mechanism.

3.2 Effect of temperature

The effect of temperature on k of the CaO powder slurry for *E. coli* was examined within the temperature range where there was no reduction in the number of viable cells. The concentration of the CaO powder slurry was 0.15 mg/ml. The temperature significantly affected the bactericidal action of the CaO powder slurry on *E. coli* (**Fig. 5**). At 42°C, *E. coli* at the concentration of about 10^6 CFU/ml was completely killed within 5 min. Even when the slurry temperature was changed, the death of *E. coli* in the CaO powder slurry followed a first-order reaction kinetics.

Figure 6 shows the Arrhenius plot of the bactericidal action of the CaO powder slurry on *E. coli*. It is noticeable that the slope of the Arrhenius plot changed at approximately 22°C. Two values of the activation energy (E_a) were obtained from the following equation (Table 1).

$$k = A \exp((-E_a/(RT)) \qquad (3)$$

In *E. coli*, the membrane functions such as the substrate transport, and the activities of the membrane-associated enzymes and the maintenance of cell integrity depend on the membrane fluidity. It seems likely that the temperature of growth and heating

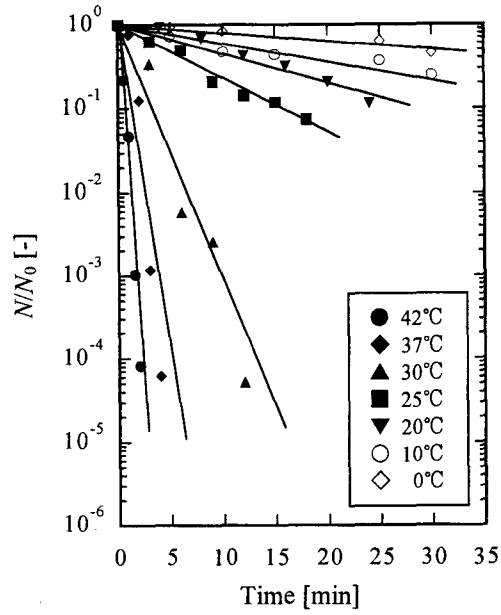

Fig. 5 Effect of slurry temperature of CaO powder on bactericidal action

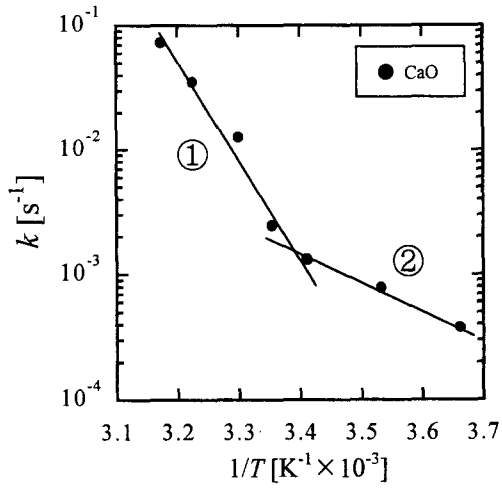

Fig. 6 k vs. $1/T$

Table 1 Values of activation energy required for death of *E. coli*

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

temperature influence the membrane fluidity. When the heating temperature of cells is above a critical level, a gel-liquid crystalline phase transition of their membrane phospholipids should occur[18]. Sinensky showed that the temperature of the phase transition of membrane lipids in *E. coli* was usually 21 to 23°C for the cell grown at 37°C [19]. The discontinuous point in Fig. 6 is almost the same as the temperature of the phase transition. Therefore, the membrane fluidity might affect the bactericidal action of the CaO powder.

Acknowledgement

A part of this work was partially supported by the Grant-in-Aid for Encouragement of Young Scientists from the Ministry of Education, Science, Sports and Culture.

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(Received December 16, 1999; Accepted May 6, 2000)