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Note

Kinetic Analysis of the Bactericidal Action of Magnesium Oxide Powder Slurry against *Escherichia coli*

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The death of *Escherichia coli* in magnesium oxide (MgO) powder slurry was found to follow first-order reaction kinetics. The value of the apparent death rate constant (k) increased with the powder slurry concentration and the slurry temperature. The bactericidal action of the MgO powder slurry was greater than that of a NaOH solution of an identical pH. *E. coli* grown at lower temperatures (T_G) became more sensitive to MgO. The Arrhenius plots of k for *E. coli* grown at 27, 37 and 45℃ exhibited discontinuous points (T_d) at approximately 13, 21 and 29 ℃, respectively, and the differences, $T_G - T_d$, were observed to be almost constant (14-16℃).

Key words : Magnesium oxide/Antibacterial activity/Bactericidal action/*Escherichia coli*/Growth temperature.

In recent years, the use of inorganic antimicrobial agents for microbial control has attracted interest (Nakashima et al., 2001; Wilczynski, 2000) . Most inorganic antimicrobial agents have immobilized antimicrobial metals and metallic salts, such as silver and copper, on inorganic materials such as ceramics, clay minerals, and activated carbon. The characteristics of inorganic materials significantly influence the amount of the immobilized agent and the release of antimicrobial metals (Ohashi and Oya, 1993; Wang et al., 1998). There is also great interest at present in inorganic materials with inherent antimicrobial activity (Bari et al., 1999; Okouchi et al., 1998).

The present authors have evaluated the antibacterial activity of 26 types of ceramic powder by the measurement of conductivity with bacterial metabolism and growth (conductimetric assay). Ten of them were found to inhibit bacterial growth. Among these active types of powder, alkaline earth metallic oxides, MgO and calcium oxide (CaO), exhibited a strong antibacterial activity (Sawai et al., 1995). CaO and MgO are generally produced by heating calcium carbonate ($CaCO_3$) and magnesium carbonate ($MgCO_3$), respectively, which are distributed widely in the environment. The main components of shells, such as oyster and scallop shells, and natural minerals, such as dolomite and calcite, are $CaCO_3$ and $MgCO_3$. They change to CaO and MgO by heat treatment, both of which exhibit antibacterial activity (Sawai et al., 2000a and 2001a). The use of these metallic oxides, therefore, is expected to be useful for expanding the shelf life of foodstuff in food processing (Bari et al., 2002; Sawai et al., 2001b). In addition, when exposed to open air, CaO and MgO absorb CO_2 and return to $CaCO_3$ and $MgCO_3$. CaO and MgO are very safe, and are expected to be utilized in natural environmental preservation as well as food processing. However, there have been few quantitative studies on the antibacterial activity of these materials (Okouchi et al., 1998; Sawai et al., 2001c), and no kinetic study on the antimicrobial activity of MgO has been made. In this study, we analyzed kinetically the bactericidal action of MgO powder slurry against *Escherichia coli*.

E. coli 745 was obtained from the Tokyo Metropolitan Research Laboratory of Public Health. The bacteria stored at −80℃ were thawed and preincubated with Brain Heart Infusion broth (Eiken Chemicals, Tokyo, Japan) at the growth temperatures of 27, 37 and 45℃ for 20 h. The cells were washed

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once with sterile distilled water and resuspended in the distilled water at a final concentration of approximately 10^8 cfu/ml. *E. coli* cells after 20h preincubation at each temperature was checked to be in a stationary phase. The bacterial suspension was kept in iced water before use in the experiments.

MgO powder (Wako Pure Chemicals, Osaka, Japan) was heated at 180 ℃ for 20min and stored in a desiccator. The mean particle size of the MgO powder was 3.6 μm. The powder was suspended in distilled water to yield the specified concentration. A 20ml aliquot of the powder slurry was poured into a glass vessel (inner diameter: 32mm) and agitated using a magnetic stirrer at 250rpm. The slurry temperature was controlled using a water bath. The bacterial suspension (0.1ml) was pipetted into the slurry. From time to time, a sample (0.1ml) was withdrawn and diluted using saline cooled in ice water. The diluted samples were pour-plated on Nutrient Agar plates (Eiken Chemicals). Triplicate plates were used for each dilution. The colonies were counted after incubation at 37℃ for 48h.

Figure 1 shows the bactericidal action of MgO powder slurry against *E. coli* grown at 37℃. The slurry temperature was also 37℃. The ordinate is the survival ratio of bacteria cfu post-treatment (N) to the non-treated cfu (N_0). The initial concentration of the viable cells (N_0) was approximately 10^6cfu/ml. An increase in the concentration of the MgO powder slurry enhanced the bactericidal action against *E. coli*. Assuming that the death of *E. coli* by the MgO powder follows first-order reaction kinetics, as given by Eq. 1, the first-order death rate constant, k (s^{-1}), of *E. coli* by MgO powder slurry can be determined.

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

where, t is treatment time. Linearity was obtained between k and the powder concentration as shown in Fig. 2. The following relationship between k and the agent concentration (C) is well known (Yanagida, 1980).

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

where α is an empirical constant and n is the dilution coefficient representing the dependence of k on C. The obtained value of n and α for *E. coli* grown at 37 ℃ was 0.44 and 1.04×10^{-3} at a slurry temperature of 37℃, respectively.

MgO hydrates to be magnesium hydroxide [$Mg(OH)_2$], which dissolved slightly [$Mg(OH)_2 \rightarrow Mg^{2+} + 2OH^-$]. The effect of Mg^{2+} on the survival of *E. coli* cells was examined. The solubility of $Mg(OH)_2$ is 1.68×10^{-4} mol/l ($=6.8\times10^{-3}$ g/l) (Annonymous, 1981). A $MgCl_2$ solution containing Mg^{2+} at the concentration of the solubility of $Mg(OH)_2$ did not affect the survival ratio of *E. coli* (data not shown).

Then, the *E. coli* used in the present study was examined for alkali tolerance, because the MgO powder slurry had a pH of approximately 10.4 (Table 1). A NaOH solution (pH10.0−11.0) of 20 ml was poured into the vessel to examine the alkaline tolerance of *E.*

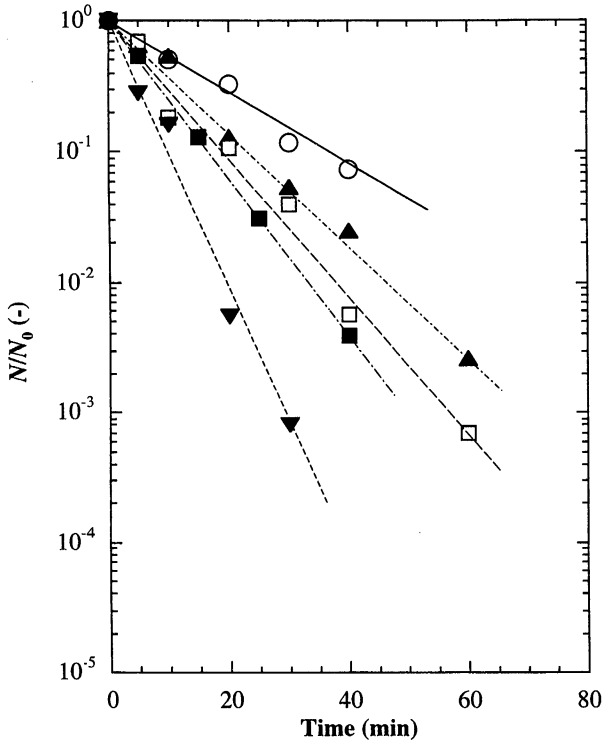

FIG. 1. Death curves for *E. coli* grown at 37℃ for treatment with MgO powder slurry at 37℃. Symbols: ○, 1.25 mg/ml; ▲, 2.5 mg/ml; □, 5 mg/ml; ■, 10 mg/ml; ▼, 20 mg/ml.

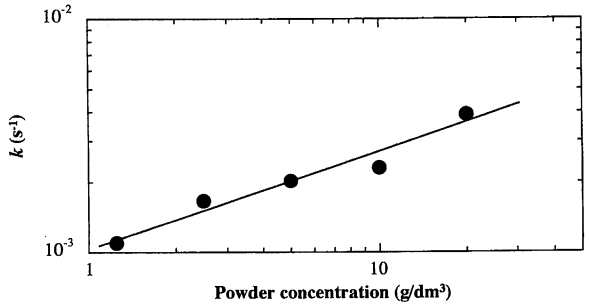

FIG. 2. Death rate constant for treatment with MgO powder slurry at 37℃ for *E. coli* grown at 37℃.

TABLE 1. Alkali tolerance of *E. coli* 745 grown at 37℃.

pH	Incubation time (min)				
	0	10	25	40	60
10.5	1.00[a]	0.87	0.79	0.76	0.73
10.75	1.00	0.40	0.34	0.28	0.25
11.0	1.00	0.13	0.034	—[b]	—

[a]Survival ratio
[b]Not done

coli. The bacterial suspension of 0.1 ml was then pipetted into the NaOH solution, and the number of viable cells of *E. coli* in NaOH solution was determined. The survival ratio of *E. coli* 745 in a NaOH solution of pH10.5 only decreased to 0.75 after a 60 min exposure, indicating that the MgO powder slurry has a much greater bactericidal action than the alkaline effect alone. In a previous study (Sawai et al., 2000b), injuries of bacterial cells caused by the MgO powder slurry were studied on the basis of a change in sensitivity to four kinds of antibiotics (penicillin G, chloramphenicol, nalidixic acid and rifampicin). *E. coli* cells treated by MgO powder slurry became sensitive to chloramphenicol and rifampicin. Although the pH of the MgO powder slurry is high, the tendency of changes in antibiotic sensitivities caused by alkaline treatment were obviously different from those caused by MgO powder slurry. Alkaline treatment did not increase any sensitivity to the four antibiotics. These results for alkaline treatment agreed with those reported by Mendonca et al. (1994). Therefore, the MgO powder slurry obviously has an antibacterial factor in addition to the alkaline effect. The generation of active oxygen such as superoxide anions was observed from MgO powder slurry by chemiluminescence analysis (Sawai et al., 1996). Some sensitivity changes in response to MgO were in agreement with those induced by active oxygen (Sawai et al., 2000b). Recently, Hewitt et al. (2001) supported our results for the bactericidal action of MgO and CaO powders with their flow cytometry results. Also, Hayashi et al. (2002) reported that a material containing CaO, which is an alkaline earth metallic oxide as is MgO, was prepared by the solid-state reaction of a mixture of calcium carbonate ($CaCO_3$) and alumina (γ-Al_2O_3) and that it was observed by ESR measurement to generate active oxygen species, such as O^- and O_2^-. Although the mechanism of active oxygen generation from MgO has yet to be understood clearly, active oxygen may also related to the antibacterial activity in addition to alkalinity.

The effect of the slurry temperature on the k values of the MgO powder slurry for *E. coli* was examined in the temperature range where there is no reduction in the viability of *E. coli* cells. Figure 3 shows the results for the treatment of *E. coli* grown at 37℃ with MgO powder slurry at 5.0mg/ml. Temperature was seen to significantly affect the bactericidal action of the MgO powder slurry on *E. coli*. The death of *E. coli* was again assumed to follow first-order reaction kinetics with varying slurry temperatures. For *E. coli* grown at 37℃, it is noticeable that the slope of the Arrhenius plot changes at approximately 22℃ (Fig. 4). The activation energy (E_a) required for death of *E. coli* by

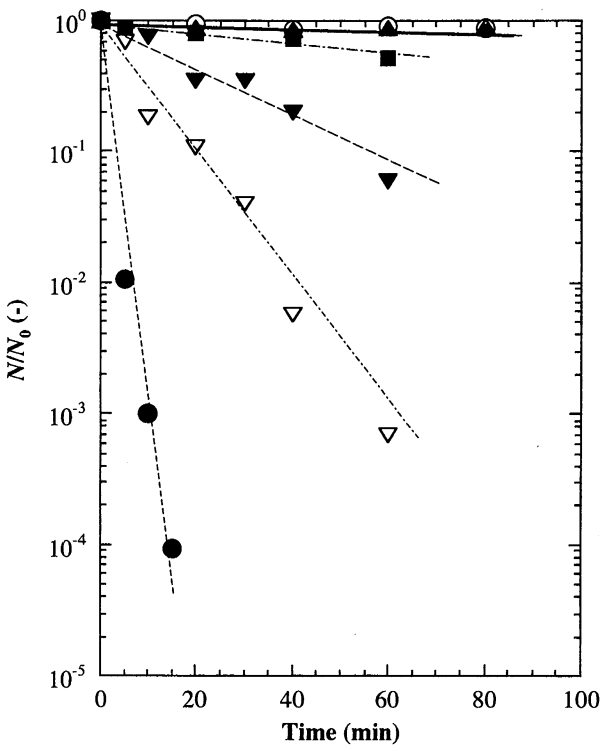

FIG. 3. Effect of temperature on the antibacterial activity of MgO powder slurry (5.0mg/ml) against *E. coli* grown at 37 ℃. Symbols: ○, 5 ℃; △, 10 ℃; ▲, 15 ℃; ■, 25 ℃; ▼, 30 ℃; ▽, 37℃;● 45℃.

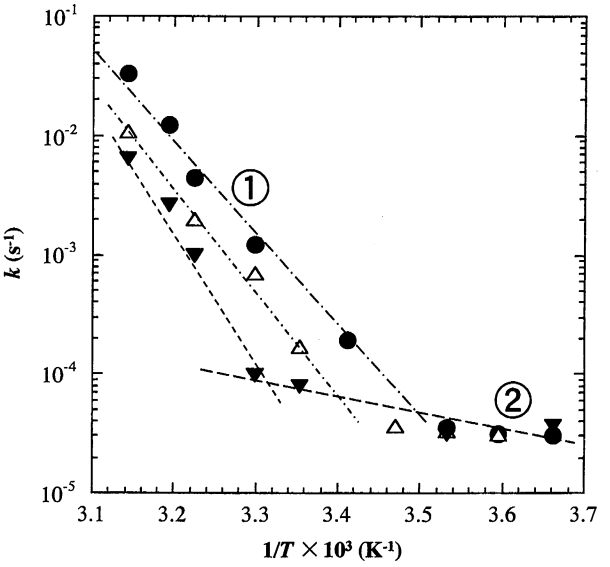

FIG. 4. Arrhenius plots of death rate constants in the treatment with MgO powder slurry (5.0mg/ml) of *E. coli* grown at different temperatures. Symbols:●, 25℃; △, 37℃; ▼, 45 ℃; ①, phase ①; ②, phase ②.

MgO was obtained from the following equation (Table 2).

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

where A, R and T are the frequency factor (s^{-1}), gas

TABLE 2. Activation energy and frequency factor for *E. coli* grown at different temperatures.

Growth temperature, T_G(℃)	T_d(℃)	$T_G - T_D$(℃)	Phase	E_a(J/mol)	A(s^{-1})
27	12.8	14.2	①	1.60×10^5	4.65×10^{24}
37	20.5	16.5	①	1.59×10^5	1.31×10^{24}
45	29	16	①	2.28×10^5	2.40×10^{35}
27, 37, 45			②	2.57×10^4	2.13×10^0

constant (J/mol• K) and slurry temperature (K), respectively. The value of E_a declined markedly from approximately 10^5 to 10^4 J/mol at discontinuous temperature points (T_d). The same experiments were then conducted for *E. coli* grown at 27 and 45℃. As shown in Fig. 4, *E. coli* grown at lower temperatures became more sensitive to MgO. *E. coli* grown at both 27 and 45℃ also had a discontinuous temperature points (T_d) in the Arrhenius plot, approximately at 13 and 29 ℃, respectively.

In bacteria, the membrane functions such as substrate transport, activities of the membrane-associated enzymes, and maintenance of cell integrity depend on membrane fluidity. It appears likely that the temperature of growth and the exposure temperature influence the membrane fluidity. When the heating temperature of the cells is above a critical level, a gel-liquid crystalline phase transition of the membrane phospholipids should occur (Katsui et al., 1981). Sinensky (1974) showed that the temperature of the phase transition of membrane lipids in *E. coli* was usually 14 to 16℃ below T_G. A decrease in T_G causes an increase in the proportion of unsaturated fatty acids in membrane phospholipids of *E. coli* (Yatvin, 1977), resulting in a change in the temperature of the phase transition. As shown in Table 2, the difference between T_G and T_d was approximately 14 to 16℃, identical to that between T_G and the temperature of the phase transition proposed by Sinensky (1974). Therefore, the significant change in the value of E_a at T_d for *E. coli* grown at different temperatures would be related to the phase transition of cell membranes. At the temperatures lower than that of the phase transition, the lipids of the cell membrane are in a gel state with low fluidity, and the normal functions of membrane are not fulfilled (Fendler, 1982). The changes in the lipid composition and fluidity of the cell membrane bring about a change in membrane permeability and alter the conformation of the charged molecules, such as proteins on the surfaces of cells. This may cause a variation in the interaction between the cells and the powder.

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