Change of Antibacterial Activity with Oxidation of ZnS Powder

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硫化亜鉛粉末の酸化に伴う抗菌活性の変化

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The antibacterial activities of samples prepared from oxidation of ZnS powder were studied by measuring the change in electrical conductivity with the growth of *Escherichia coli*. Oxidation of ZnS occurred at above 550°C in air and then the amount of ZnO increased with increasing the oxidizing temperature. The obtained crystal structure of ZnO was a zinc-blende type. From results of antibacterial tests on ZnS powder samples before and after oxidation, the antibacterial activity was found to increase with increasing the oxidizing temperature. However, no activity of ZnS was observed, irrespective of the powder concentration. The pH value after dispersing the powder samples in physical saline solution was in the range between 5.5 and 8.0, increasing with the formation of ZnO. [Received December 21, 1998; Accepted June 21, 1999]

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1. Introduction

Microbial pollution and contamination which took place by microorganisms, have been produced various problems in industrial and vital fields, such as degradation and infection, etc. In order to solve these problems, therefore, new sterilization and antibacterial techniques have been demanded and studied.¹⁾⁻⁴⁾

Recently, the occurrence of antibacterial activity by using ceramic powders has been pointed out much attention as new technique substituting for conventional organic agents with the activity.⁵⁾⁻⁸⁾ On the ceramics powder with the activity, three ceramic powders of zinc oxide (ZnO), calcium oxide (CaO) and magnesium oxide (MgO) were found to show a remarkable antibacterial activity. The use of three ceramics presented has the advantages following as; containing mineral element essential to human body, showing a strong activity in a small amount of ceramic powder and without the irradiation of light.⁹⁾⁻¹¹⁾ However, it was not clarified whether or not the oxygen in their ceramics is concerned as the chemical species on antibacterial activity.

In the case of ZnO, the crystal structure and the electrical properties are similar to those on ZnS. For example, ZnO and ZnS show the electrical conductivity of n-type and give the crystal structure of either wurtzite- or zincblende-type. Therefore, to use ZnS powder is essential to examine the contribution of oxygen in ZnO on antibacterial activity.

In the present work, the powder samples used in antibacterial tests were prepared with and without oxidizing ZnS at various temperatures in air. After preparing the slurries of the samples obtained, the evaluation of the antibacterial activity was carried out by measuring the change in electrical conductivity with the growth of *Escherichia coli*.

2. Experimental

2.1 Preparation of precursor samples and test bacteria

ZnS powder (purity: 99.99%) was used as a starting material, which was shown in Fig. 1. ZnS powder with a particle size of approximately 0.5 μm was heated at each temperature in air with the heating rate of 10°C/min. The sample powders heated were suspended with physiological saline in the concentration of 1.6 to 100 mg/ml and then the

slurries prepared were used in antibacterial tests.

In order to know the crystal structure and the chemical composition of the powder samples prepared, X-ray diffraction measurement (XRD) and energy-dispersive X-ray measurement (EDX) was carried out, respectively. The thermal decomposition processes of ZnS were determined by thermogravimetry-differential analysis (TG-DTA).

Escherichia coli 745 (*E. coli*) was used as a test bacterium and stored at Tokyo Metropolitan Research Laboratory of Public Health. *E. coli* was cultured in Brain Heart Infusion (BHI) broth at 37°C for 24 h on a reciprocal shaker. The bacterial culture was suspended in a sterile physiological saline with a final concentration of approximately 10^3 CFU/ml.

2.2 Test of antibacterial activity

The evaluation of the antibacterial activity of powder samples was carried out by measuring the change in electrical conductivity with the growth of *E. coli* (conductance method). Bactometer microbial monitoring system model 64 was used as the apparatus for measuring the conductivity. The preparation of bacteria into the wells of a module for the Bactometer was carried out following as; adding the sample slurries into the well containing modified plate count agar (MPCA) and then dispensing the bacterial suspension into the well. After setting the module in Bac-

Fig. 1. SEM micrograph of ZnS powder used in antibacterial tests.

tometer, the change of electrical conductivity was monitored during incubation at 37°C for 20 h in dark place. These procedures were reported in detail.¹⁰⁾

In order to examine indirectly the pH values when the sample slurries were added into the well, the powder samples with and without oxidation were dispersed into physiological saline with the powder concentration of 0.3 to 5.0 mg/ml. After keeping the dispersed solutions for 24 h, the pH values of solutions were measured.

3. Results and discussion

3.1 Thermal decomposition of ZnS

The thermal decomposition of ZnS was measured by TG-DTA with the heating rate of 10°C/min and shown in Fig. 2. An exothermic peak with weight loss was observed at 660°C, starting at 550°C and ending at 700°C, and also a slight weight loss without endo- and exo-thermic peaks was confirmed in the ranging from 700 to 830°C. The overall weight loss of approximately 16.2% approached the theoretical one of ZnO produced due to the oxidation of ZnS, which were in agreement with the results reported by Schultze et al.¹²⁾ And also, they discussed the oxidation process of ZnS by analyzing XRD patterns recorded *in situ* during the thermal oxidation. The overall reaction for thermal oxidation was written as followings:

Assuming that the reactions (1) and (2) occurred in this study, the exothermal weight gain should be observed as addition of oxygen to ZnS yielding either ZnSO₄ or Zn₃O(SO₄)₂. However, no weight gain was observed and also no diffraction peaks corresponding to ZnSO₄ and Zn₃O(SO₄)₂ were detected from the results of XRD measurement described below. From the result described above, it was suggested that the oxidation of ZnS without their intermediates started rapidly at 550°C.

In Fig. 3, XRD patterns are shown on the samples oxidized at each temperature in air. Below the oxidizing temperature of 400°C, only diffraction peaks corresponding to ZnS gave only. By the oxidation of 600°C, however, small peaks corresponding to ZnO were detected in addition to those of ZnS, suggesting the oxidation of small amount of ZnS. At 900°C, the peaks of ZnS were observed slightly and the diffraction intensity of ZnO became strongly. It may suggest the formation of large amount of ZnO.

The chemical compositions of raw material (ZnS) and

Fig. 3. XRD patterns of the samples oxidized at each temperature in air.

Table 1. Chemical Compositions of the Samples Oxidized at 400, 600 and 900°C, and Together with ZnS

element (mol%)	ZnS	oxidizing temp. (°C)		
		400	600	900
S	40.49	42.10	39.32	3.74
Zn	59.51	57.90	60.68	96.26
other	not detected	not detected	not detected	not detected

the samples oxidized at the temperature ranging from 400 to 900°C were measured by EDX and summarized in Table 1. The concentration of sulfur in raw material was similar to those on the sample oxidized at 400°C. At the oxidation above 600°C, the concentration of sulfur in the samples decreased with the increase of the temperature, due to the oxidation of ZnS. These results agreed with that of XRD measurement described above.

3.2 Change of electrical conductivity with bacterial growth

With the growth of bacteria, *E. coli*, it was known that the electrolytes such as organic acid and amino acid were produced with the digestion of proteins in medium.¹³⁾ The electrical conductivity in growth medium increases with the increase of the electrolytes produced. Therefore, it makes possible to evaluate the antibacterial activity. And also, the change of conductivity occurs when the bacterial concentration in medium reaches approximately 10⁷ CFU/ml.

By using the powder samples of ZnS with and without oxidation, the evaluation of antibacterial activity was carried out by measuring the electrical conductivity with the growth of *E. coli*.

Figures 4(a), (b), (c) and (d) show the change of electrical conductivity in the case of ZnS and the sample heated at either 400, 600 or 900°C, respectively. In these figures, the incubation time to detect the change is called, "Detection Time," DT. Hence, if the value of DT is delayed by adding the powder samples, it can be distinguished that the samples have the effect of a growth inhibition on the bacteria. In the case of ZnS powder, the DT value was approximately 8 h, irrespective of the powder concentration. As the value was comparable with that without the addition of powder samples, no antibacterial activity of ZnS was found in the powder concentration ranging from 1.6 to 100

Fig. 2. TG-DTA curve of ZnS powder with the heating rate of 10°C/min.

Fig. 4. The change in electrical conductivity of the samples against *E. coli*:

(a) ZnS powder, the powder sample oxidized at (b) 400, (c) 600 and (d) 900°C.

Broken line means the change in electrical conductivity of the samples in the case of the absence of *E. coli*.

mg/ml. On the other hand, the DT value of sample oxidized at either 400, 600 or 900°C increased with the increase of powder concentration and even the delay of the DT value was found with the increase of the oxidizing temperature. From these results, it was found that the antibacterial activity against *E. coli* increased with increasing the concentration of ZnO.

3.3 Comparison of antibacterial activity

Figure 5 shows the comparison of antibacterial activity of three samples oxidized at 400, 600 and 900°C, and together with ZnS. The vertical axis, "DT/DT_{cont.}," represents the ratio of the DT value at specified concentration of samples to that at no addition of samples (cont.). If the value of DT/DT_{cont.} is changed with a steep rise at lower powder concentration, it can be evaluated to show a stronger antibacterial activity. As shown in Fig. 5, ZnS showed a constant in the value of DT/DT_{cont.}. With the increase of the oxidizing temperature on ZnS, the pronounced change of the value was found at lower powder concentration. In other words, it was clarified the oxidation of ZnS resulted in an effective antibacterial activity on *E. coli*.

The growth inhibition of bacteria by ceramic powders may be pointed out to be concerned with four factors following as:¹⁴⁾ (1) the metal ions eluted from ceramic powders, (2) the active oxygen generated from ceramic powders, (3) the effect of pH value in solution dispersing ceramic powder and (4) the mechanical destruction of cell membrane to be in contact with ceramic powders. However, Yamamoto et al.¹⁰⁾ and Sawai et al.¹⁵⁾ reported that no effect of metal ions

Fig. 5. Comparison of antibacterial activity against *E. coli*.

against antibacterial activity was observed. Therefore, we supposed that the appearance of activity is due to the mechanical destruction, pH value and the active oxygen.

As a first step, the mechanical destruction of cell membrane was measured by stirring a sterile physiological saline with a concentration of approximately 10^3 CFU/ml dispersing Al_2O_3 powder (average particle size: $1.0 \mu\text{m}$) without antibacterial activity,¹⁴⁾ the powder concentration of Al_2O_3 being 100 mg/ml. In Fig. 6, survival ratio of *E. coli*

Fig. 6. Survival ratio of *E. coli* with stirring time; the used powder being Al_2O_3 with a particle size of $1\ \mu\text{m}$.

with stirring time at the rotation speed of 400 rpm was shown. As shown in this figure, no survival ratio with rotation time during 1 h decreased, i.e., no bactericidal action by mechanical effect. This result suggests that no appearance of antibacterial activity is responsible for the mechanical destruction of cell membrane by ceramic powder.

The pH value in physiological saline dispersing ZnO powder has been known to be in the ranging from 7.0 to 8.0,⁶⁾ no appearance of the activity being observed in the pH region. In this study, ZnS powder with and without oxidation was used in antibacterial tests. Therefore, it is essential to examine the pH value in physiological saline dispersing powder samples. As a secondary step, the pH values in physiological saline dispersing powder samples were measured.

In Fig. 7, the values of pH in physiological saline were shown. In the case of the samples oxidized at above 600°C , the values were detected in the range of 7.5 to 8.0. However, ZnS and powder sample oxidized at 400°C showed the value of acidic region, suggesting the generation of sulfuric acid due to the elution of sulfur from the surface of sample powder. However, the pH value in the observed acidic region did not affect the bacterial growth.

From these results, it was supposed that the pH value and the mechanical effect did not affect antibacterial activity in all powder samples. The appearance of antibacterial activity of ZnO has been considered to be due to the generation of H_2O_2 from the surface of ZnO powder.^{6),10),15)} On the powder sample oxidized at 900°C , hydrogen peroxide, H_2O_2 , was measured with the same operation of previous paper.¹⁰⁾ H_2O_2 was detected in a concentration of $5\ \mu\text{g}/\text{ml}$, when the powder concentration in physiological saline was $50\ \text{mg}/\text{ml}$. From the result and the reports, the increase in the antibacterial activity with the oxidation of ZnS seems to originate from the increase of H_2O_2 accompanied with increasing the amount of ZnO.

Fig. 7. The pH values in physiological saline dispersing the samples with and without oxidations.

4. Conclusion

By using the ZnS powder oxidized at various temperatures, the evaluation of the antibacterial activity was carried out by measuring the change in electrical conductivity with the growth of *Escherichia coli*. The following results were found.

The oxidation reaction of ZnS occurred above 550°C and then the concentration of sulfur in the samples decreased with the increase of the oxidizing temperature.

The antibacterial activity of the oxidized ZnS against *Escherichia coli* was found; the activity being shown in the increase of the powder concentration and the oxidizing temperature, i.e., increase of an amount of ZnO. However, no antibacterial activity of ZnS without oxidation was detected.

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