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Antibacterial Activity of Products obtained by Oxidation of ZnS

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Abstract: By using the products prepared with the oxidation of ZnS powder, the antibacterial activities were studied by measuring the change in the electrical conductivity with the growth of *Escherichia coli*. The oxidation reaction of ZnS occurred above 550℃ in air and then an amount of ZnO increased with an increase of the oxidizing temperature. The crystal structure of ZnO deposited was of zincblende-type. From the results of antibacterial tests, the antibacterial activity increased with the increase of the oxidizing temperature. However, no activity of ZnS was observed, irrespective of the powder concentration. The value of pH in physiological saline containing the powder samples obtained was shown in the ranging from 5.5 to 8.0, which was found to increase with the formation of ZnO.

Key words : Zinc oxide, Zinc sulfide, Oxidation, Antibacterial activity, Conductance method.

1. Introduction

Microbial pollution and contamination that took place by microorganisms, have produced various problems in industrial and vital fields, such as degradation and infection, etc. In order to solve these problems, therefore, new pasteurization and antibacterial techniques have been demanded and studied [1-3].

Recently, the occurrence of antibacterial activity by using ceramic powders has been pointed out with much attention as a new technique substituting for conventional techniques using organic agents with the activity [4-6]. On the ceramics powders, zinc oxide (ZnO), calcium oxide (CaO) and magnesium oxide (MgO) were found to show a remarkable antibacterial activity. The use of the three ceramics has the following advantages; containing mineral elements essential to human body, showing a strong activity in a small amount of ceramic powder and without the irradiation of light [7-9]. 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 samples used in antibacterial tests were prepared by oxidizing ZnS at various temperatures in air. After preparing aqueous slurries of the samples obtained, the evaluation of the antibacterial activity was carried out by measuring the change in electrical conductivity of the slurries 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. ZnS powder was heated at various temperatures in air with a heating rate of 10℃/min. Thus obtained sample powders were suspended with physiological saline in the concentration of 1.6 to 100 mg/ml, and then the slurries 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) were 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 Heat Infusion (BHI) at 37℃ for 24h 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 Bactmeter was carried out as follows; adding the sample slurries into the well containing Modified plate count ager (MPCA) and then dispensing the bacterial suspension into the well. After setting the module in Bactometer, the change of electrical conductivity was monitored during incubation at 37℃ for 20h in dark place. These procedures were reported in detail [8].

3. Results and Discussion

3.1 Thermal decomposition of ZnS

The thermal decomposition of ZnS was measured by TG-DTA with a heating rate of 10 ℃ /min and shown in Fig. 1.

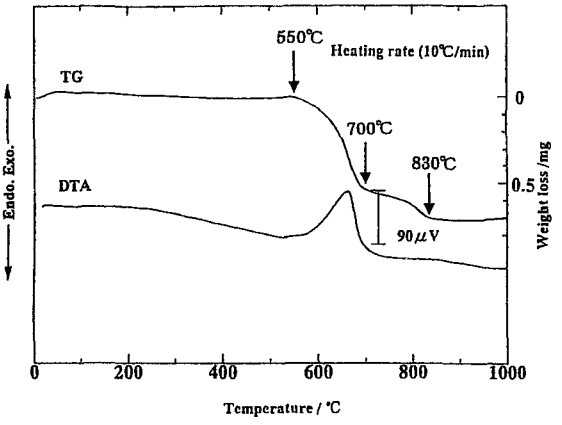

Fig.1 TG-DTA curve of ZnS powder with a heating rate of 10℃/min.

An exothermic peak with a weight loss was observed at 660℃, (starting at 550℃, ending at 700℃), and also a slight weight loss without endo- and exo-thermic peaks was confirmed in the ranging from 700 to 830℃. The overall weight loss of approximately 16.2% approached the theoretical one due to the formation of ZnO by the oxidation of ZnS, which were in agreement with the results reported by D. Schultze, *et. al.* [10]. 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 follows:

$$ZnS + 2O_2 \rightarrow ZnSO_4 \quad (1)$$

$$3ZnSO_4 \rightarrow Zn_3O(SO_4)_2 + SO_3 \quad (2)$$

$$Zn_3O(SO_4)_2 \rightarrow 3ZnO + 2SO_3 \quad (3)$$

Assuming the reaction (1) and (2), an exothermal weight gain should be observed as the addition of oxygen to ZnS yields either $ZnSO_4$ or $Zn_3O(SO_4)_2$. However, no weight gain was observed and also no XRD diffraction peaks corresponding to $ZnSO_4$ and $Zn_3O(SO_4)_2$ were detected as described below. Consequently, it was found that the oxidation reaction of ZnS in this study started rapidly at 550℃.

Figure 2 shows XRD patterns of the samples oxidized at various temperatures in air. Below 400℃, all diffraction peaks are assigned to those of ZnS. By the oxidation at 600℃, small peaks corresponding to ZnO were detected in addition to those of ZnS. The formation of ZnO suggests the oxidation of a little amount of ZnS. At 900℃, XRD diffraction peaks of ZnS were observed slightly and the diffraction of ZnO became strongly instead.

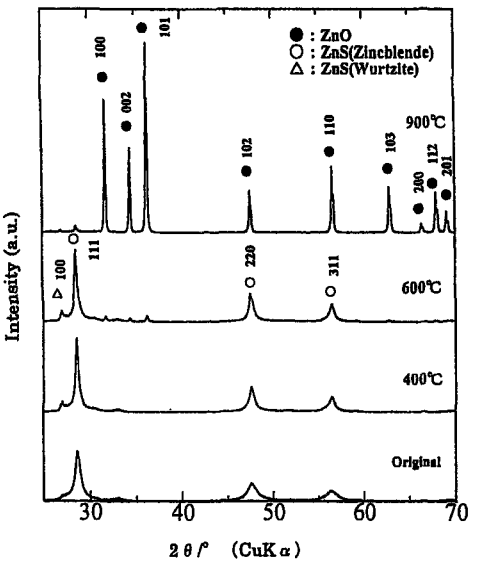

Fig. 2 XRD patterns of the samples oxidized at various temperatures in air.

The chemical compositions of the raw material (ZnS) and the samples oxidized at temperatures ranging from 400 to 900℃ were measured by EDX (Table 1).

Table 1 Chemical compositions of the samples oxidized at 400, 600 and 900℃, and together with ZnS.

	Original	400℃	600℃	900℃
S(mol%)	40.49	42.10	39.32	3.74
Zn(mol%)	59.51	57.90	60.68	96.26

The concentration of sulfur in the raw material was similar to those on the sample oxidized at 400℃. At the oxidation temperatures above 600℃, the concentration of sulfur in the samples decreased with increases in temperature, *i.e.*, due to the oxidation of ZnS.

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 acids and amino acids were produced with the digestion of proteins in medium [11]. The electrical conductivity in such a growth medium increases with the increase of the electrolytes produced. Actually, the change of conductivity occurs when the bacterial concentration in medium reaches approximately 10^7 CFU/ml.

The evaluation of antibacterial activities of ZnS with and without heating in air was carried out by measuring the electrical conductivity with the growth of *E. coli*.

Figure 3 (a) and (b) show the change of electrical conductivity in the case of ZnS and the sample heated at 900℃, respectively.

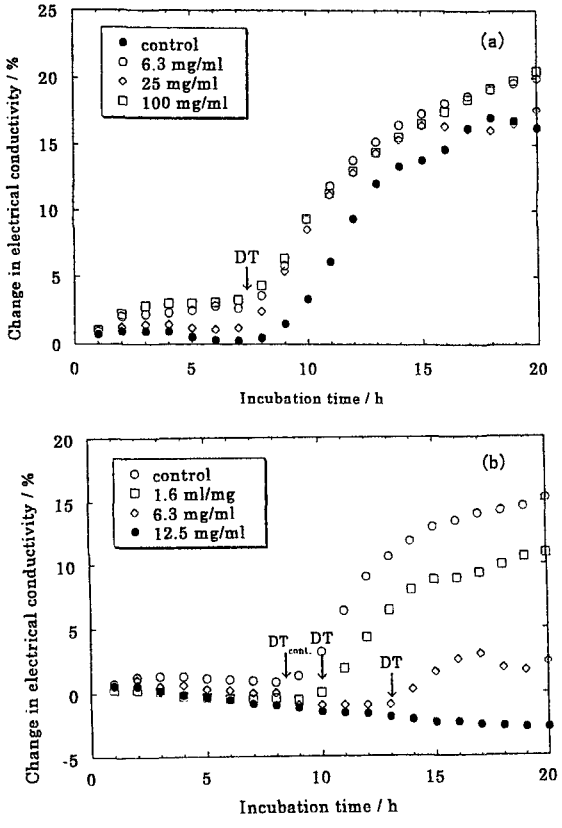

Fig. 3 Changes in electrical conductivity of the samples against *E. coli*: (a) ZnS powder, (b) the powder sample oxidized at 900℃.

Here, DT (Detection Time) is the incubation time at which an electrical change is detected. 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 (see Fig. 3(a)), the DT value was approximately 8h, irrespective of the powder concentration. As the DT value was comparable with that in the case of no additive of powder samples, no antibacterial activity of ZnS was observed in the powder concentration ranging from 1.6 to 100 mg/ml. On the other hand, the DT value of the sample obtained at 900℃ increased with the increase of powder concentration (Fig. 3(b)), which meant the increase in antibacterial activity against *E. coli* by increasing the concentration of ZnO in medium.

3.3 Comparison of antibacterial activity

Figure 4 shows the comparison of antibacterial activity of ZnS and three samples oxidized at 400, 600 and 900℃.

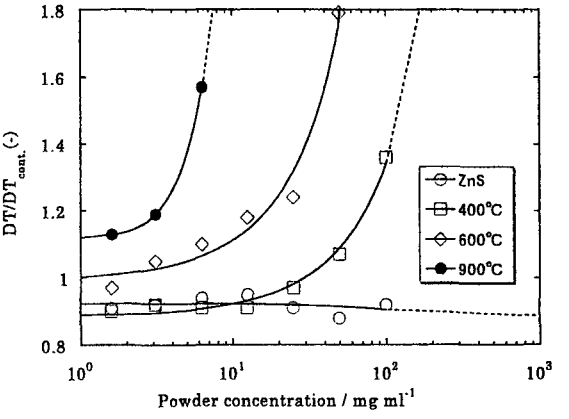

Fig. 4 Comparison of antibacterial activity against *E. coli*.

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 $DT/DT_{cont.}$ ratio is changed with a steep rise at lower powder concentration, it can be evaluated to show a stronger antibacterial activity. As shown in Fig. 4, ZnS showed a constant in the value of $DT/DT_{cont.}$ ratio. With the increase of the oxidizing temperature for ZnS, the pronounced change of the value was found at lower powder concentrations. In other words, the oxidation of ZnS resulted in an effective antibacterial activity on *E. coli*.

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 [8, 12]. And also, the generation of H_2O_2 has been observed around neutrality. Consequently, the pH values in physiological saline dispersing the powder samples were measured. The pH values increased with the increase of the oxidizing temperature; the pH values for the samples oxidized at 600 and 900℃ being 7.5 and 8.0, respectively. From these results, it was suggested that the antibacterial activity appeared to be due to the generation of H_2O_2.

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℃ 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.*, the increase of the amount of ZnO. However, no antibacterial activity of ZnS without oxidation was detected.

References

1. T. Lusaka and Y. Takagi, *J. Antibact. Antifung. Agents*, **20**, 451-457(1992).
2. M. Saito, *J. Antibact. Antifung. Agents*, **21**, 17-21(1993).
3. Y. Tsunoda, H. Egawa and O. Yuge, *J. Antibact. Antifung. Agents*, **20**, 571-575(1992).
4. J. Sawai, H. Igarashi, A. Hashimoto, T. Kokugan and M. Shimizu, *J. Chem. Eng. Jpn*, **28**, 288-293(1995).
5. J. Sawai, H. Igarashi, A. Hashimoto, T. Kokugan and M. Shimizu, *J. Chem. Eng. Jpn*, **28**, 556-561(1995).
6. J. Sawai, H. Igarashi, A. Hashimoto, T. Kokugan and M. Shimizu, *J. Chem. Eng. Jpn*, **29**, 251-256(1996).
7. J. Sawai, O. Yamamoto, M. Hotta, H. Kojima and T. Sasamoto, *J. Chem. Soc. Jpn*, **1998**, 633-636(1998).
8. O. Yamamoto, M. Hotta, J. Sawai, T. Sasamoto and H. Kojima, *J. Ceram. Soc. Jpn*, **106**, 1007-1011(1998).
9. O. Yamamoto, J. Sawai, M. Hotta, H. Kojima and T. Sasamoto, *J. Ceram. Soc. Jpn*, **106**, 1252-1254(1998).
10. D. Schultze, U. Steinike, J. Kussin and U. Kretzschmar, *Cryt. Res. Technol.*, **30**, 553-558(1995).
11. J. C. S. Richards, A. C. Jason, G. Hobbs, D. M. Gibson and R. H. Christie, *J. Phys. E: Sci. Instrum.*, **11**, 560-568(1978).
12. J. Sawai, H. Kojima, H. Igarashi, A. Hashimoto, S. Shoji, T. Kokugan and M. Shimizu, *J. Ferment. Bioeng.*, **86**, 521-522(1998).

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