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Effect of lattice constant of zinc oxide on antibacterial characteristics

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Zinc oxide powders were heated in different atmospheres at 800 and 1400 °C, of which the characterization and the antibacterial activity were studied by X-ray diffractometry and the measurement of the change in electrical conductivity with bacterial growth. The diffraction peaks corresponding to zinc oxide with hexagonal type structure were detected in all samples, which shifted in low-angle side with the increase in the oxidizability of atmosphere during heat-treatment. From the results of calculating lattice constants, a_0 and c_0, it was found that the value of c_0 in hexagonal structure increased with the increase in the oxidizability of atmosphere. On the samples heated at 1400 °C, the changes of the c_0 value were less than those at 800 °C. However, no change of the a_0 value showed, irrespective of atmosphere and temperature. Hydrogen peroxide that contributes to the occurrence of antibacterial activity was found to generate from all samples, and the generation amount increased with the increase of c_0 value; incidently the amount in the samples heated at 1400 °C was less than that at 800 °C. The antibacterial activity of zinc oxide increased with the increase of c_0 value; that is, it was found that the value of c_0 in crystal structure affected the antibacterial activity of zinc oxide.

1. Introduction

In industries and other vital fields, microbial contaminations that take place by microorganisms have produced various problems [1, 2]. In order to avoid the bacteria that are harmful to humans from water, some organic compounds, such as quarternary ammonium salt and chlorine disinfectant have been used as conventional antibacterial agents [3, 4].

Recently, the occurrence of antibacterial activity on ceramic powders, such as calcium oxide (CaO), mangesium oxide (MgO) and zinc oxide (ZnO), has been pointed out with much attention as a novel technique that can substitute for conventional organic agents [5–12]. Among these ceramic powders described above, we have recommended the utilization of ZnO as an antibacterial agent for the reason that ZnO has been found to have several advantages: ZnO shows a marked antibacterial activity in neutral region (pH = 7) without the presence of light and zinc is a mineral element essential to the humans, etc. [13–15]. Hydrogen peroxide (H_2O_2) was generated from the surface of ZnO crystal, which was effective for the inhibition of bacterial growth [16, 17]. In our previous reports, however, it was not clear how change in antibacterial activity is expected by the difference of ZnO structure, such as lattice constant, and the structure affects the generation of H_2O_2 that contributed in the occurrence of antibacterial activity.

The aim of the present work is to evaluate antibacterial activity of ZnO as a function of lattice constant of hexagonal structure via H_2O_2 amount generated.

2. Materials and methods

2.1. Preparation of samples and test bacteria

ZnO powder (purity: 99.95%, average diameter: 0.2 μm, Kanto Chemical Co., Inc.) was used as a starting material and heated for 1 h at either 800 or 1400 °C in different atmospheres, such as H_2, air, O_2 and $O_2 + 4\,\%\,O_3$. The obtained powder samples were suspended with physiological saline in the concentration range from 1.6×10^3 to $1.0 \times 10^5\ \mathrm{g\,m^{-3}}$ and then the resultant slurries were used in antibacterial tests. In order to examine lattice constant, X-ray diffraction measurement (XRD; RIGAKU, RTNT-2500VHF) was carried out in all samples. The morphological aspect of particles was observed by a scanning electron microscope (SEM; JEOL, JXA840).

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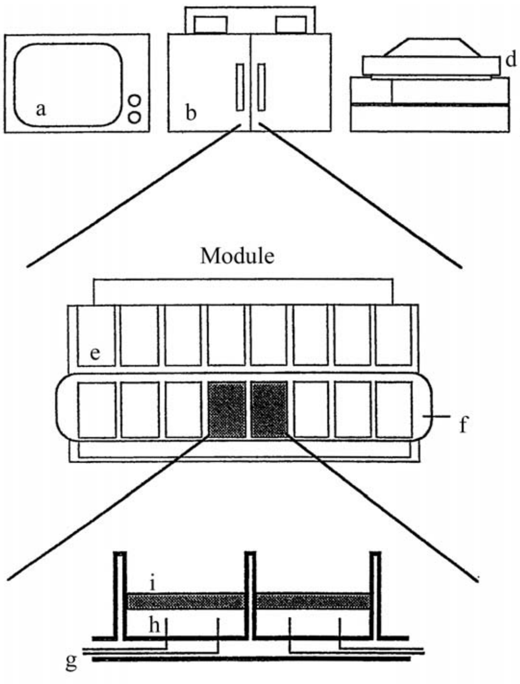

a. Display
b. BPU (Bactometer Processing Unit)
c. Computer
d. Printer
e. Well
f. Cap
g. Electrodes
h. Modified Plate Count Agar
i. Bacterial suspension and powder slurry

Figure 1 Schematic illustration of the apparatus used in the antibacterial tests.

Escherichia coli IFO 3301 (*E. coli*) was used as a test bacterium. The bacterium was cultured in a brain heat infusion broth (BHI; Eiken Chemical Co.) at 37 °C for 24 h with shaking on a reciprocal shaker. The bacterial culture was suspended in a sterile physiological saline at a final concentration of about $10^2 \mathrm{CFU\,m^{-3}}$ (CFU; colony-forming unit).

2.2. Tests of antibacterial activity

Antibacterial activity of powder samples was evaluated by measuring the change in electrical conductivity with bacterial growth. The apparatus for measuring the electrical conductivity was Bactometer Microbial Monitoring System model 64 (BMMS; bioMerieux-VITEK), as shown in Fig. 1. In BMMS, the electrodes exist at the bottom of the sample well. Preparation of *E. coli* into the wells of a module for the Bactometer was carried out as follows; putting the powder slurries into the well containing a modified plate count agar (MPCA; Difco Co.) and then adding the bacterial suspension into the well. After setting the module in the Bactometer, the change in electrical conductivity was monitored during incubation at 37 °C for 30 h without the presence of light.

2.3. pH measurement

The pH value in the medium has been known to affect antibacterial activity; that is, the activity occurs in the pH region below 3 and above 10 [21]. In order to examine the pH values when the powder slurries were added into the well, powder samples were dispersed into physiological saline at a powder concentration of about $25 \times 10^3 \mathrm{\,g\,m^{-3}}$. After keeping the dispersed solutions for 24 h, the pH value of physiological saline was measured by a pH meter (model PH81, Yokogawa Electric Co.).

2.4. Analysis of hydrogen peroxide generated from sample

Using spectrophotofluorometer (SPF; SHIMAZU, RF-5000), the quantitative analysis of H_2O_2 generated from ZnO was carried out as follows: H_2O_2 was detected by using 10-Acetyl-3, 7-dihydroxyphenoxazine (Amplex™ Red: Molecular Probes, Inc.) [18]. In a presence of horseradish peroxidase (HRP; Sigma Chemicals Co., Ltd.), Amplex™ Red reacts with H_2O_2 to produce highly fluorescent resorufin, which shows an excitation maximum at 563 nm and an emission maximum at 587 nm. $1 \times 10^{-6} \mathrm{\,m^3}$ of the water containing H_2O_2 was added to $2 \times 10^{-6} \mathrm{\,m^3}$ of $100 \mathrm{\,mol\,m^{-3}}$ phosphate buffer ($\mathrm{pH} = 7.0$) containing $127 \mathrm{\,g\,m^{-3}}$ Amplex™ Red and $150 \mathrm{\,g\,m^{-3}}$ HRP. The mixture was incubated at 37 °C for 15 min, and then $5 \times 10^{-7} \mathrm{\,m^3}$ of $100 \mathrm{\,g\,m^{-3}}$ sodium dodecylsulfate (SDS; Wako Pure Ind., Ltd.) was added into the mixture. The H_2O_2 standard solutions between 3.15×10^{-3} and $31.5 \times 10^{-3} \mathrm{\,g\,m^{-3}}$ were produced by diluting $3.15 \times 10^5 \mathrm{\,g\,m^{-3}}$ H_2O_2 solution, in order to carry out the calibration of generated H_2O_2. The concentration of generated H_2O_2 was measured by absorbance of H_2O_2 ($\varepsilon_{240} = 0.128 \mathrm{\,m^2\,g^{-1}}$) at 240 nm [19].

3. Results and discussion

3.1. Powder samples

Sample code, heat-treatment conditions, average particle size and specific surface area of the samples used in this study are summerized in Table I. As shown in Table I, irrespective of atmosphere, the average particle size and the specific surface area of samples was about 0.8 μm and about $5.1 \mathrm{\,m^2\,g^{-1}}$ at 800 °C, and about 4.5 μm and about $1.1 \mathrm{\,m^2\,g^{-1}}$ at 1400 °C, respectively. X-ray diffraction peaks observed on all powder samples could be indexed on the base of ZnO with hexagonal system, and these peaks shifted to low-angle side on the samples heated in O_2 and $O_2 + 4\% \, O_3$, irrespective of temperature. Table II

TABLE I Sample code, particle size and specific surface area of ZnO used in this work

Sample code	Temperature (°C)	Atmosphere	Particle size (μm)	Specific surface area ($\mathrm{m^2\,g^{-1}}$)
H-800	800	H_2	0.8	5.2
A-800	800	Air	0.8	5.1
O-800	800	O_2	0.8	5.1
O′-800	800	$O_2 + 4\% \, O_3$	0.8	5.1
A-1400	1400	Air	4.5	1.1
O-1400	1400	O_2	4.5	1.1
O′-1400	1400	$O_2 + 4\% \, O_3$	4.5	1.1

TABLE II Lattice constant of ZnO heated in different atmospheres at 800 and 1400 °C

Sample code	Temperature (°C)	Atmosphere	Lattice constant (nm)	
			Along a_0	Along c_0
H800	800	H_2	0.3250	0.5207
A800	800	Air	0.3250	0.5207
O800	800	O_2	0.3250	0.5210
O′800	800	$O_2 + 4\% O_3$	0.3250	0.5215
A1400	1400	Air	0.3250	0.5207
O1400	1400	O_2	0.3250	0.5208
O′1400	1400	$O_2 + 4\% O_3$	0.3250	0.5209

summarizes the results of calculating lattice constants, a_0 and c_0, from the diffraction peaks detected XRD. On the samples heated at 800 °C, the value of c_0 axis in hexagonal structure changed from 0.5207 to 0.5215 nm depending on atmosphere, although the a_0 axis was a constant value of 0.3250 nm. At 1400 °C, the changes of the c_0 value were less than those at 800 °C. That is, the value of c_0 axis in hexagonal system was found to increase with the increase in oxidizability of atmosphere during heat-treatment. Since ZnO has been known to be nonstoichiometric crystals, having excess zinc, the change of lattice constant may be related to the presence of interstitial zinc ion [20]. Then, we examine a molar ratio (Zn/O) in ZnO by chelatometry using 8-quinolinol (Kishida Chemical. Co.). However, the molar ratio of all samples was 1 within 10^{-4} order accuracy; that is, it was not possible to find the difference in chemical composition among powder samples. Therefore, the reason for the increase in the value of c_0 axis with the increase in the oxidizability of atmosphere during heat-treatment is not yet clear.

3.2. Amount of generated H_2O_2

The amount of H_2O_2 generated from powder samples is summarized in Table III. At 800 °C, the H_2O_2 amount generated from H800 sample was similar to those from A800 sample. In O800 and O′800 samples, however, the amount of H_2O_2 generated was larger than in H800 and A800 samples. At 1400 °C, the effect of oxidizability on the amount of H_2O_2 generated was similar to those at 800 °C, though the amount of H_2O_2 was less than at 800 °C. As compared with Table II, the good correlation is observed between the value of c_0 axis and the amount of H_2O_2 generated, that is, the amount of H_2O_2 increases with the increase of c_0 value in ZnO hexagonal system. Based on the discussion above, the increase of H_2O_2 amount is assumed to be due to the increase in the oxidizability of atmosphere during the heat-treatment of powder sample, that is, the increase of the value of c_0 axis in hexagonal structure.

TABLE III Amount of generated H_2O_2 on the samples heated in different atmospheres at 800 and 1400 °C

Sample code	Amount of generated H_2O_2 (10^3 g m^{-3})
H800	15.99
A800	15.98
O800	19.95
O′800	32.62
A1400	4.31
O1400	5.48
O′1400	8.54

3.3. Antibacterial activity of ZnO

Regarding growth of the bacteria, it is known that electrolytes such as organic and amino acids are produced with the digestion of hydrocarbons and proteins in the medium [22]. The electrical conductivity in such a growth medium, therefore, increases with increasing amount of electrolytes produced; the conductivity changes occurring at a bacterial concentration of about 10^7 CFU m^{-3} in the medium.

Fig. 2 shows the changes in electrical conductivity with incubation time for *E. coli* in the slurries with different concentrations of the sample heated at 800 °C in $O_2 + 4\% O_3$ (O′800). The symbol of detection time (DT) indicates the incubation time at which an electrical change can be detected. Hence, when the value of DT is delayed by adding the powder sample, it can be concluded that the sample has the effect of inhibiting bacterial growth. In the case where no powder sample was added (control), the DT value was approximately 8 h. On adding sample, however, the DT value increased with increasing powder concentration and no DT value could be detected at a powder concentration of 25.0×10^3 g m^{-3}. This indicates an increase in antibacterial activity with the increase in powder concentration in the medium.

Based on the change in electrical conductivity described above, the antibacterial activity of all powder samples was examined for *E. coli*.

Fig. 3(a) and (b) compare the antibacterial activity of powder samples heated at 800 and 1400 °C. The vertical axis, ''DT/DT$_{\text{control}}$'', represents the ratio of the DT values at specified powder concentrations to that for no powder sample addition (control). If the values of DT/DT$_{\text{control}}$ change with a steep rise at the lower powder concentrations, the powder sample can be understood to have the stronger antibacterial activity. In samples heated at 800 °C (see Fig. 3(a)), with the decrease in oxidizability of the atmosphere during heat-

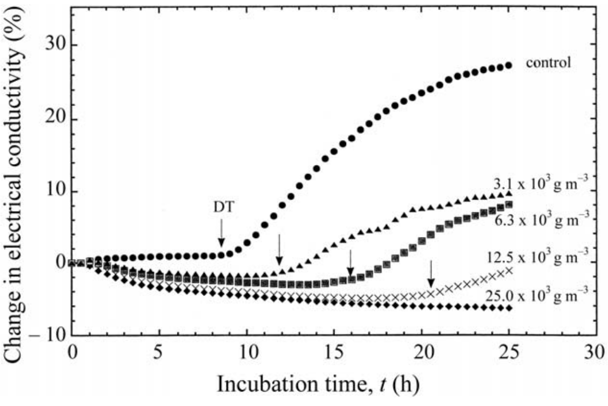

Figure 2 The changes in electrical conductivity with incubation time of *E. coli*.

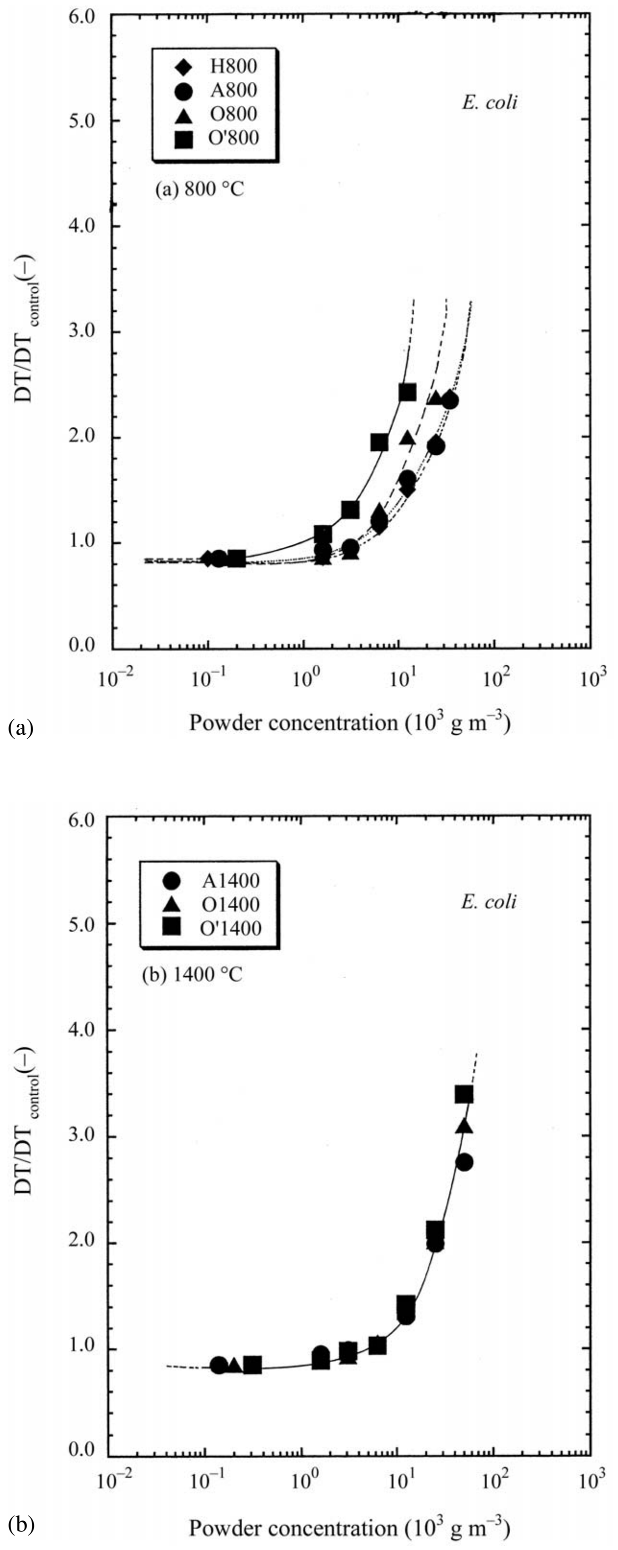

Figure 3 The comparison in the antibacterial activity for *E. coli* on the samples heated in different atmospheres at (a) 800 °C and (b) 1400 °C.

treatment, a marked change of the value was found at higher powder concentration; that is, the antibacterial activity of O′800 sample was stronger than those of O800 sample, and the activity of A800 and H800 samples was weaker than those of O′800 and O800 samples. The antibacterial activity of A800 sample was comparable with those of H800 sample. On other word, at 800 °C, it can be presumed that the antibacterial activity of sample increased with the increase of c_0 value in hexagonal structure of ZnO. With respect to samples heated at 1400 °C (see Fig. 3(b)), a change in the $\mathrm{DT/DT_{control}}$ value occurred at an identical powder concentration, irrespective of atmosphere; that is, the effect of atmosphere on the antibacterial activity of samples obtained at 1400 °C was less than that of samples heated at 800 °C. This reason is presumed to the low sensitivity of *E. coli* in the case that the amount of H_2O_2 in medium was low. And also, the activity of ZnO obatined at 1400 °C was found to be weaker than that at 800 °C. Yamamoto [13] reported that the antibacterial activity of ZnO with a small particle size was stronger than that with a large particle size. The reason was presumed as follows: the contact of moisture in the medium per unit ZnO mass increased with the decrease in particle size, because of the increase of specific surface area. It resulted in the increase of H_2O_2 amount generated from its surface. In the present work, the specific surface area from ZnO obtained at 1400 °C was smaller than that at 800 °C as shown in Table I. The reason that the antibacterial activity of ZnO obtained at 1400 °C was weaker than that at 800 °C was presumed to be a small amount of H_2O_2 generated from the surface of ZnO. In other words, the c_0 value in hexagonal structure and the specific surface area of ZnO affected the increase in antibacterial activity.

On *E. coli* used in antibacterial test, Yamamoto *et al.* [14, 16] and Sawai *et al.* [17, 23] reported that the effect of pH on antibacterial activity of ZnO could be not recognized within pH = 5.5 ∼ 8.0. In order to examine the pH values when the powder samples were dispersed in physiological saline, the pH value of physiological saline was measured by a pH meter. In the result, it was found that the value was 7.5 for all powder samples. Therefore, it was presumed that pH did not affect the occurrence of antibacterial activity.

4. Conclusion

ZnO powders were heated in different atmospheres at 800 and 1400 °C, and antibacterial activity of ZnO as a function of lattice constant of hexagonal structure via H_2O_2 amount generated was studied. The following conclusions can be drawn from this study:

1. The diffraction peaks corresponding to ZnO were detected in all powder samples, and the peaks shifted to low-angle side on the samples heated at 800 °C in O_2 and $O_2 + 4\,\%\, O_3$. By calculating lattice constants, a_0 and c_0, it was found that the value of c_0 in hexagonal structure changed from 0.5207 to 0.5215 nm. However, no change of a_0 value showed, irrespective of atmosphere and temperature. In the case of the samples at 1400 °C, the change of a_0 value was smaller than at 800 °C.
2. The generation of H_2O_2 was found in all powder samples. The amount of generated H_2O_2 was found to be increased with the increase in the value of c_0 axis in hexagonal structure. In comparison with 800 and 1400 °C, it was found that the amount of H_2O_2 on the samples heated at 800 °C was larger than that on the samples obtained at 1400 °C.
3. In the samples heated at 800 °C, the antibacterial activity increased with the increase in the value of c_0 axis in hexagonal structure. At 1400 °C, there was no effect of c_0 value on the activity. In comparison with the antibacterial acitivity between 800 and 1400 °C, it was

found that the activity of the samples obtained at 1400 °C was weaker than those at 800 °C.

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