Activated Carbon Sphere with Antibacterial Characteristics

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The activated carbon sphere containing zinc oxide was prepared by carbonizing a zinc ion-exchange resin at different temperatures in nitrogen gas. Zinc oxide of hexagonal type was detected in all carbon samples, the amount of which decreased with an increase in the carbonization temperature. However, the specific surface areas of carbon samples increased with increasing temperature of the resin. The antibacterial activity on their carbon samples was studied without the presence of light. The antibacterial activity on carbon samples containing zinc oxide increased with the amount of zinc oxide in the carbon samples. The antibacterial activity for *Staphylococcus aureus* was stronger than that for *Escherichia coli*. By an oxygen electrode analysis, it is shown that hydrogen peroxide was generated on the carbon samples. The concentration of hydrogen peroxide increased with increasing carbonization temperature of the resin. The antibacterial activity is found to be caused by the generation of hydrogen peroxide from zinc oxide dispersed in activated carbon sphere.

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1. Introduction

Microbial pollution and contamination that take place by microorganisms have produced various problems in industry and other vital fields, including medicine and infection, etc.^{1,2)} Organic compounds such as quarternary ammonium salts and chlorine disinfectant have been used as conventional antibacterial agents for the elimination of microbial contamination,³⁾ but noxious materials in the human body may be included in the organic agent.⁴⁾ Therefore, novel pasteurization and antibacterial treatments have been required.

Recently, the occurrence of antibacterial activity on ceramic powders has been pointed out with much attention as a new technique that can substitute for conventional ones using organic agents.⁵⁻⁷⁾ On ceramic powders with antibacterial activity, zinc oxide has been found to show a marked antibacterial activity in the absence of light,⁸⁻¹²⁾ and the antibacterial ability has been noticed. The use of zinc oxide as an antibacterial agent also has the following advantages: it shows strong antibacterial activity under the pH values in the range from 7 to 8, and zinc is a mineral element essential to the human body. The occurrence of antibacterial activity has been supposed to be due to the generation of active oxygen from its surface.¹⁰⁾ However, it is known that the activity by active oxygen weakens with lengthening the diffusion distance of active oxygen until it reaches bacteria.

Yamamoto *et al.* reported that activated carbons were excellent in affinity with microorganisms and adsorbed large amount of bacteria.¹³⁾ Therefore, zinc oxide deposited in the activated carbon is anticipated to have pronounced antibacterial activity in the water containing bacteria, because of the short diffusion distance of active oxygen that reaches the bacteria. Assuming that pathological bacteria included in drinking water can be decontaminated by packing activated carbon containing zinc oxide into an ion-exchange column, the mate-

rial is possible to use as a novel antibacterial agents to remove bacterial pollution.

Spherical activated carbons containing zinc oxide were prepared through the carbonization of resins ion-exchanged by zinc ion. The aim of the present work is to study the antibacterial activity of the obtained activated carbons and to quantitatively analyze active oxygen contributed in the activity.

2. Experimental Procedure

2.1 Preparation of carbon sphere

An ion-exchange resin (WK10: Mitsubishi Chemical, Co.) having a carboxyl group as exchangeable function group was used as a starting material. A resin with a particle size of about 0.5 mm was treated for 24 h by an aqueous solution containing $[\text{Zn}(\text{NH}_3)_2]^{2+}$ complex. The amount of zinc ion in the treated resin was about 4.6 mol kg^{-1} . The ion-exchanged resins were carbonized for 10 min in a high-purity nitrogen gas at temperatures ranging from 500 to 900°C to prepare activated carbons containing zinc oxide. The thus-obtained carbon samples with zinc oxide were suspended into physiological saline at concentrations ranging from 1.6×10^{-3} to $1.0 \times 10^{-1} \text{ g m}^{-3}$. They were used in antibacterial tests.

2.2 Characterization of carbon samples

The formation of zinc oxide in carbon samples was examined by X-ray diffraction measurements (XRD: RIGAKU, RINT-2500 VHF). The shape of the carbon samples was observed by a scanning electron microscope (SEM: JEOL, JXA840). In order to examine the distribution of zinc oxide in carbon samples, an energy dispersive x-ray analyzer (EDX) installed on the SEM was used on the cross-section of carbon samples. The amount of zinc oxide in the samples was determined by oxidizing at 900°C in air. The specific surface areas of the samples were estimated by analyzing the adsorption isotherms of nitrogen gas at -196°C (BET: Bell Japan Inc., BELSORP 28).

In order to examine the pH values in bacterial growth, the carbon samples were dispersed into physiological saline at a concentration of $12.5 \times 10^{-3} \text{ g m}^{-3}$. After keeping the dis-

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persed solution for 24 h, the pH values of physiological saline were measured.

2.3 Antibacterial tests

Escherichia coli 745 (hereafter, *E. coli*) and *Staphylococcus aureus* 9779 (hereafter, *S. aureus*) were used as test bacteria. These bacteria were cultured in a brain heart 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 1.0×10^{-4} CFU m^{-3} (CFU: Colony Forming Unit).

By measuring the changes in the electrical conductivity with bacterial growth, we assessed the antibacterial activity of carbon samples.^{10,12)} The apparatus for measuring the conductivity was a Bactometer Microbial Monitoring System Model 64 (bioMeieux), as shown in Fig. 1. The electrodes exist at the bottom of the sample well, and the potential between the electrodes is 84 mV. Placing the bacteria into the wells of a module of the Bactometer was carried out as follows: the carbon samples were placed in a well containing a modified plate count agar (MPCA: Difco, Co) and then the bacterium suspension was dispensed into the well. After setting the module in the Bactometer, the change in the electrical conductivity was monitored during incubation at 37°C for 30 h in the absence of light.

2.4 Analysis of hydrogen peroxide

After dispersing the prepared activated carbons into physiological saline at different powder concentrations, the active oxygen in the saline, such as hydrogen peroxide (H_2O_2), was measured by an oxygen electrode. The detection of H_2O_2 by oxygen electrode is possible to detect by using enzymes, such as catalase. Because the $\text{H}_2\text{O}_2 \rightarrow \text{H}_2\text{O} + 1/2\text{O}_2$ reaction occurs by adding catalase into physiological saline, the concentration of H_2O_2 generated can be calculated from the concentration of O_2 detected by the oxygen electrode. In order to examine the generation of H_2O_2 from zinc oxide itself, zinc oxide with the particle size of 1.0 μm (ZnO: Kanto Chemical Co., purity; 99.95%) was prepared by heating at different temperatures for 1 h in air. The obtained powders were dispersed into physiological saline at a concentration of 5.0×10^{-3} g m^{-3} and the generated H_2O_2 in physiological saline was measured by the process described above.

3. Results

3.1 Characterization of activated carbon sphere

The sample code, the carbonization temperature, the zinc oxide content, the specific surface area and the pH value of activated carbons are summarized in Table 1. The amount

Fig. 1 Schematic illustration of the apparatus used in antibacterial test.

of zinc oxide in the carbon samples was about 65 mass% for CS-500, and decreased with an increase of the carbonization temperature. However, the specific surface areas of the carbon samples increased with an increase in the carbonization temperature; that is, the value increased from 201 to 523 m^2g^{-1} . The pH values in physiological saline dispersed with carbon samples were found to be 5.6–5.7.

The XRD patterns of the activated carbons containing zinc oxide are shown in Fig. 2. The diffraction peaks corresponding to zinc oxide of hexagonal type were detected in the carbon samples. With an increase in the carbonization temperature, these peaks of zinc oxide became sharper.

Figure 3 shows SEM-micrograph of a carbon sample carbonized at 900°C (CS-900). The shape was spherical with diameter of about 350 μm . The distribution of zinc oxide in the sample is shown in Fig. 4. From these observations, zinc oxide was found to exist homogeneously in the carbon sample. The shape and distribution of other samples were similar to that of CS-900.

3.2 Antibacterial effect of carbon samples

Regarding the growth of the bacteria, it is known that the electrolytes such as organic and amino acids are pro-

Table 1 Characterization of activated carbon sphere containing zinc oxide.

Sample code	Carbonization temperature (°C)	ZnO content (mass%)	Specific surface area (m^2g^{-1})	pH (—)
CS-500	500	65	201	5.7
CS-700	700	63	242	5.6
CS-900	900	52	523	5.6

Fig. 2 XRD patterns of activated carbons containing zinc oxide.

Fig. 3 SEM-micrograph of the activated carbon containing zinc oxide obtained at 900°C (CS-900).

Fig. 4 Line analysis for ZnK α of the cross-section in the activated carbon containing zinc oxide obtained at 900°C (CS-900).

duced with the digestion of hydrocarbons and proteins in the medium, and the electrical conductivity in the medium increases with bacterial growth.¹⁴⁾ The electrical conductivity in such a growth medium, therefore, increases with increasing amount of the electrolytes produced, the change occurring at a bacterial concentration of about 10 CFU m⁻³ in the medium.

Figure 5 shows the changes in the electrical conductivity with incubation time for *S. aureus* on CS-500. In the figure, DT (detection time) indicates the incubation time at which an electrical change can be detected. Hence, if the value of DT is delayed by adding the carbon samples, it can be concluded that the carbon samples have the effect of inhibiting bacterial growth, i.e., bacteriostatic effect. When no DT is detected during the incubation time, it can be judged that the carbon samples have the effect of the extinction of bacteria, i.e., bactericidal effect. In the case where no carbon sam-

Fig. 5 The change in electrical conductivity with incubation time of *Staphylococcus aureus*, in the case of the addition of the activated carbon containing zinc oxide obtained at 500°C (CS-500). The powder concentration of a, control; b, $3.1 \times 10^{-3} \text{ g m}^{-3}$; c, $6.3 \times 10^{-3} \text{ g m}^{-3}$; d, $12.5 \times 10^{-3} \text{ g m}^{-3}$.

Fig. 6 The comparison in the bactericidal effect for *Staphylococcus aureus* and *Escherichia coli*.

ples were added (control), the DT value was about 10 h. On adding carbon samples, however, the DT value increased with increasing powder concentration. The change in the DT value for *S. aureus* was similar to that for *E. coli*. The results indicate an increase in bacteriostatic effect on increasing the concentration of activated carbon containing zinc oxide in the medium; that is, the antibacterial activity increased with increasing powder concentration.

Based on the change in the electrical conductivity described above, the bactericidal effect of all carbon samples was examined for two bacteria, *S. aureus* and *E. coli*.

In Fig. 6, the bactericidal effect of three activated carbons towards *S. aureus* and *E. coli* is compared. The vertical axis represents the powder concentration at no detection of DT, i.e., bactericidal powder concentration. If the bactericidal powder concentration is small, it can be taken to show stronger bactericidal effect. As the figure shows, the bactericidal powder concentration increased linearly along with increasing the carbonization temperature. The behavior on *E. coli* was comparable with that on *S. aureus*; that is, the bactericidal effect of carbon sample obtained at 500°C is shown to be stronger than those prepared at 700 and 900°C. With respect to *E. coli*, the bactericidal powder concentrations were higher than those for *S. aureus*; that is, the antibacterial activ-

ity of the carbon samples was found to be weaker towards *E. coli* than towards *S. aureus*.

3.3 Detection of hydrogen peroxide

The concentration of hydrogen peroxide (H_2O_2) generated from the surface of carbon samples is shown in Fig. 7. The concentration of H_2O_2 increased linearly with increasing powder concentration, irrespective of the carbonization temperature. For CS-500, the H_2O_2 concentration reached about $1.2 \times 10^{-12} \text{ g m}^{-3}$ at a powder concentration of $2.0 \times 10^{-3} \text{ g m}^{-3}$. With increasing temperature, the concentration of H_2O_2 was found to decrease at a specified powder concentration. That is, the H_2O_2 concentration generated from powder samples is found to increase with decreasing carbonization temperature. In the case where zinc oxide powder was added in physiological saline (see Fig. 8), the concentration of H_2O_2 decreased with increasing the heating temperature. In other word, the generated H_2O_2 decreased with increasing

crystallinity of zinc oxide.

4. Discussion

The specific surface areas of the activated carbon sphere containing zinc oxide increased along with increasing the carbonization temperature (Table 1). Nakagawa *et al.*¹⁵⁾ prepared activated carbons by carbonizing ion-exchange resins having different cations, such as zinc, nickel and copper ions. They clarified that the formation of micropores in the carbons was due to the pillars that were formed in the molecular structure of an ion-exchanged resin *i.e.*, a pillar effect. For the present activated carbons, therefore, the increase in the specific surface areas is presumed to occur due to the pillar effect.

The amount of zinc oxide in the carbon sample obtained at 900°C was about 52 mass%, which was smaller than those in samples prepared at 500 and 700°C (Table 1). The mass loss of zinc oxide is assumed to be due to the vaporization of zinc during carbonization of its resin.

By measuring the changes in the electrical conductivity with bacterial growth, it was found that the antibacterial activity increased with increasing concentration in the activated carbons containing zinc oxide in the medium (Fig. 5). Also, the activity decreased with increasing carbonization temperature (Fig. 6).

The following three factors may affect the antibacterial activity on activated carbons containing zinc oxide: (1) the pH value in the medium, (2) zinc ion eluted from zinc oxide in activated carbons and (3) active oxygen generated from the surface of zinc oxide. The pH values in physiological saline dispersed with carbon samples were 5.6–5.7 (Table 1). Radford *et al.*¹⁶⁾ and Cole *et al.*¹⁷⁾ reported that the pH values shown in present work did not affect the bacterial growth. In other words, factor (1) may have no effect on the activity. In order to examine the effect of zinc ion on the antibacterial activity, we confirmed the effect by using a physiological saline of zinc chloride with a concentration of $1.0 \times 10^{-6} \text{ mol m}^{-3}$. From the change in the electrical conductivity with bacterial growth, it was found that the DT value was similar to that in the case of the control; that is, no effect of factor (2) was observed. By oxygen electrode analysis, H_2O_2 was detected (Fig. 7), which may contribute to the antibacterial activity, because H_2O_2 is known to be effective for antibacterial activity.¹⁸⁾ Also, Yamamoto *et al.* reported the generation of H_2O_2 from the surface of zinc oxide and considered this to be effective for the inhibition of bacterial growth.¹⁰⁾ Therefore, the occurrence of antibacterial activity is related to the generation of H_2O_2 from zinc oxide deposited in activated carbons. The concentration of H_2O_2 decreased with increasing crystallinity of zinc oxide (Fig. 8) and carbonization temperature of resin ion-exchanged by zinc ions (Fig. 7). By XRD (Fig. 2), it was found that, with increasing carbonization temperature, the crystallinity of zinc oxide in activated carbons increased, and the amount of zinc oxide decreased (Table 1). Based on the discussion described above, the decrease in the antibacterial activity with increasing carbonization temperature is attributed to a decrease in the amount and an increase in the crystallinity of zinc oxide in activated carbons, *i.e.*, a decrease in the H_2O_2 concentration generated from activated carbons.

On activated carbons containing zinc oxide, the antibacte-

Fig. 7 The change in the concentration of H_2O_2 as a function of the concentration of carbon samples.

Fig. 8 The change in the concentration of H_2O_2 as a function of the heating temperature of zinc oxide.

rial activity for *S. aureus* was stronger than that for *E. coli* (Fig. 6). The structures and chemical compositions of the cell surface of the two kinds of bacteria used in this study are quite different. That is, thin layers of lipid A, lipopolysaccharide and peptidoglycan are present on the cell surface of *E. coli*, whereas there is only a peptidoglycan layer for *S. aureus*. Sawai *et al.*¹⁹⁾ reported that the activity of H₂O₂ was stronger on *S. aureus* than on *E. coli*. They also carried out an experiment to determine whether or not the H₂O₂ generated from zinc oxide was related to the antibacterial activity by using four kinds of antibiotics.²⁰⁾ In their investigation, the changes in the sensitivity of *E. coli* to the antibiotics suggested that H₂O₂ was one of the primary factors contributing to the antibacterial activity of zinc oxide. Saito *et al.*²¹⁾ reported that the H₂O₂ generated could readily penetrate the cell wall of the bacteria. Therefore, the differences in antibacterial action towards *S. aureus* and *E. coli* are attributed to the different sensitivities towards H₂O₂.

5. Conclusion

Activated carbon sphere containing zinc oxide was prepared by carbonizing the resin-exchanged zinc ion. Zinc oxide of hexagonal type was detected in activated carbons, and the zinc oxide in activated carbons was found to exist homogeneously in the carbons. With an increase in the carbonization temperature, the specific surface area of activated carbons increased, and the amount of zinc oxide in the carbons decreased.

The antibacterial activity of activated carbons containing zinc oxide increased with a decrease in the carbonization temperature and an increase in the amount of zinc oxide in the activated carbons. From a comparison of the activity, it was found that the activity for *S. aureus* was stronger than that for *E. coli*. The generation of hydrogen peroxide was observed in physiological saline dispersed with activated carbons. The occurrence of antibacterial activity was attributed to the gen-

eration of hydrogen peroxide from the surface of zinc oxide deposited in activated carbons.

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