

Preparation and Characterization of Novel Activated Carbons with Antibacterial Function

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After ion-exchange resins were treated for 24 h by an aqueous solution of $[Zn(NH_3)_4]^{2+}$ complex, the treated resins were carbonized for 10 min in nitrogen gas at each temperature. The activated carbons with ZnO were then prepared. The antibacterial activity on their carbon samples was studied without the presence of light. ZnO of hexagonal type was detected in the carbon samples, of which the amount decreased with an increase in the carbonization temperature. However, the specific surface areas of carbon samples increased with increasing the carbonization temperature of the resin. The antibacterial activity on carbon samples containing ZnO increased with a decrease of the carbonization temperature and an increase of the amount of ZnO in the samples. From a comparison of the antibacterial activity between *Staphylococcus aureus* and *Escherichia coli*, it was found that the activity for *Staphylococcus aureus* was stronger than that for *Escherichia coli*. The concentration of hydrogen peroxide increased linearly with an increase in the amount of carbon samples. The occurrence of antibacterial activity was found to be due to the generation of hydrogen peroxide from ZnO in activated carbons.

Microbial pollution has produced various problems in industry and other vital fields.[1] During the summer in Japan, the pollution of foods and the contaminated drinking water of aircraft by pathological bacteria became topics in connection with the generation of the trouble in health. In order to solve these problems, therefore, new pasteurization and antibacterial treatments have been demanded and studied.

In almost all cases, organic compounds such quarternary ammonium salt and chlorine disinfectant have been used as conventional antibacterial agents for the elimination of microbial contamination,[2] but noxious materials in the human body may be included in the organic agent.[3] Recently, the occurrence of antibacterial activity by using ceramic powders has been pointed out with much attention as a new technique that can substitute for conventional ones using organic agents.[4–6] Ceramic powder of zinc oxide (ZnO) has been found to show a marked antibacterial activity without the presence of light.[7–9] The use of ZnO as an antibacterial agent also has the following advantages: mineral elements essential to the human body and strong antibacterial activity in small amounts. Concerning ZnO, it was found that antibacterial activity existed under the pH values in the range from 7 to 8,[9] and that the values are suitable for water used for washing and drinking. The occurrence of antibacterial activity by ZnO has been supposed to be due to the generation of active oxygen from its surface.[9] 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.[10] If a large amount of bacteria adsorbs on the surface of activated carbon, ZnO deposited on the carbon surface is anticipated to have marked antibacterial activity in the water containing bacteria, because of the short diffusion distance of active oxygen that reaches the bacteria. In order to remove the bacteria that are harmful to humans from water, therefore, we propose to use activated carbons containing ZnO as a novel antibacterial agent.

In the present work, spherical activated carbons containing ZnO were prepared through the carbonization of resins ion-exchanged by zinc ion. The antibacterial activity of the obtained carbon samples was studied.

Experimental

Preparation of Samples. 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 $[Zn(NH_3)_2]^{2+}$ complex. The amount of zinc ion in the treated resin was about 4.6 mol kg^{-1}. The resin exchanged was carbonized for 10 min in a high-purity nitrogen gas at temperatures ranging from 500 to 900 °C to prepare activated carbons containing ZnO. The thus-obtained carbon samples with ZnO were suspended with physiological saline at concentrations ranging from 1.6 to 100 mg cm^{-3}. They were used in antibacterial tests.

Characterization of Samples. The formation of ZnO in carbon samples was examined by X-ray diffraction measurements (XRD: RINT-2500 VHF). The shape of the carbon samples was observed by a scanning electron microscope (SEM: JXA840). The amount of ZnO in the samples was determined by oxidizing at carbonization temperature in air. The specific surface areas of the samples were estimated by analyzing the adsorption isotherms of nitrogen gas at −196 °C (BET: 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 mg cm^{-3}. After keeping the dispersed solution for 24 h, the pH values of physiological saline were measured.

Antibacterial Tests. *Staphylococcus aureus* 9779 (*S. aureus*) and *Escherichia coli* 745 (*E. coli*) were used as test bacteria. These bacteria were 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 CFU cm^{-3}.

The antibacterial activity of carbon samples was evaluated by measuring the changes in the electrical conductivity with bacterial growth.[9,11] The apparatus for measuring the conductivity was the Bactometer Microbial Monitoring System Model 64 (bioMeieux). In this apparatus, the electrodes exist at the bottom of the sample well, and the potential between electrodes is 84 mV. The preparation of bacteria into the wells of a module for the Bactometer was carried out as follows: adding the carbon samples into the well containing a modified plate count agar (MPCA: Difco, Co.) and then giving the bacteria suspension 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 without the presence of light.

Analysis of Active Oxygen. After the prepared activated carbons were dispersed into physiological saline at different powder concentrations, the active oxygen, such hydrogen peroxide (H_2O_2), in the saline was measured by an oxygen electrode. The oxygen electrode can detect the dissolved oxygen in solution, but not directly measure H_2O_2. In the analysis by oxygen electrode, however, H_2O_2 is possible to detect by using enzymes, such as catalase. Because the $H_2O_2 \rightarrow H_2O + 1/2\ O_2$ 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.

Table 1. Characterization of Activated Carbons Containing ZnO Used in This Study

Carbonization temperature / °C	ZnO content / wt%	Specific surface area / $m^2\ g^{-1}$	pH
500	65	201	5.7
700	63	242	5.6
900	52	523	5.6

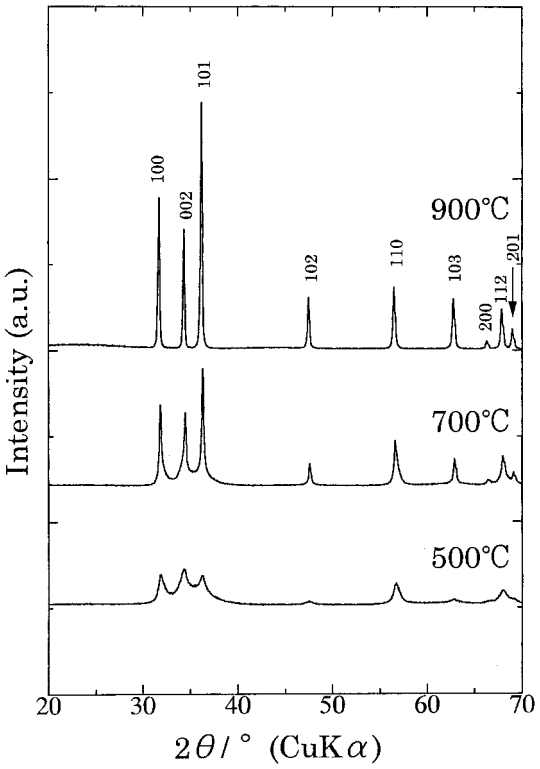

Fig. 1. XRD patterns of activated carbons containing ZnO.

Results

The Characterization of Activated Carbons Containing ZnO. The ZnO content, the specific surface area and the pH value of activated carbons used in this study are summarized in Table 1. The amount of ZnO in the carbon samples when ion-exchanged resins were carbonized at 500 °C gave about 65 wt%, and decreased with an increase of the carbonization temperature. However, the specific surface areas of the carbon samples increased along with an increase in the carbonization temperature; that is, the value increased in the range from 201 to 523 $m^2\ g^{-1}$. The pH values in physiological saline dispersed with carbon samples were found to be 5.6 and 5.7.

The XRD patterns of the activated carbons containing ZnO are shown in Fig. 1. On the carbon samples, only the diffrac-

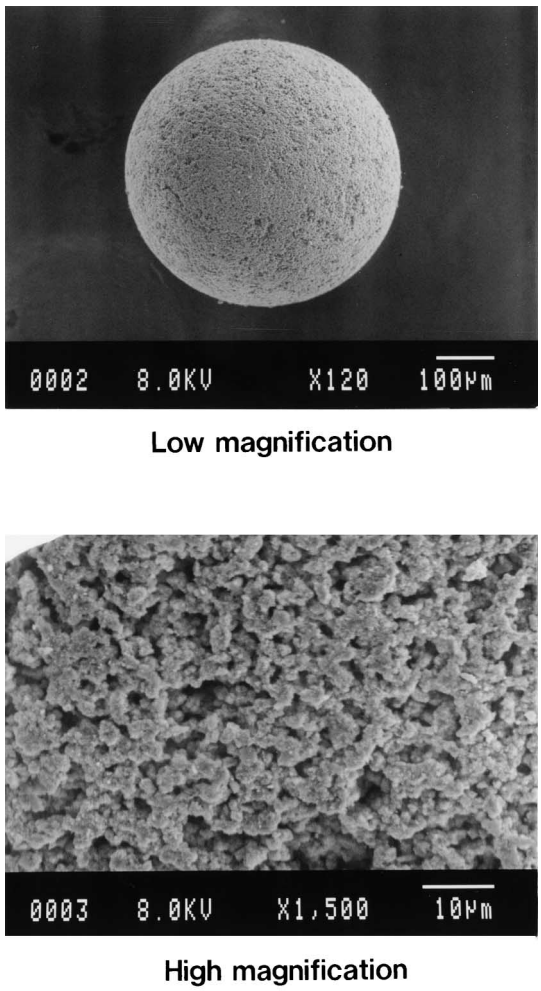

Fig. 2. SEM-micrograph of the activated carbon containing ZnO obtained at 500 °C.

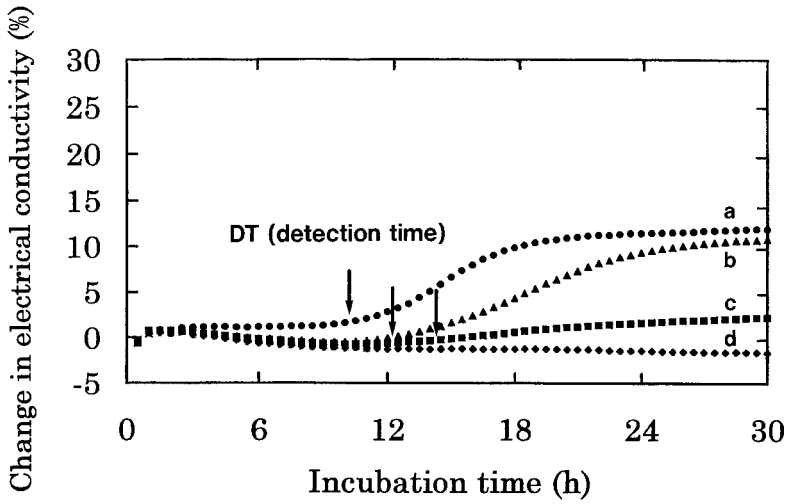

Fig. 3. The changes in electrical conductivity with incubation time of *Staphylococcus aureus*, in the case of the addition of the activated carbon containing ZnO obtained at 500 °C. The powder concentration of a, control; b, 3.6 mg cm^{-3}; c, 6.3 mg cm^{-3}; d, 12.5 mg cm^{-3}.

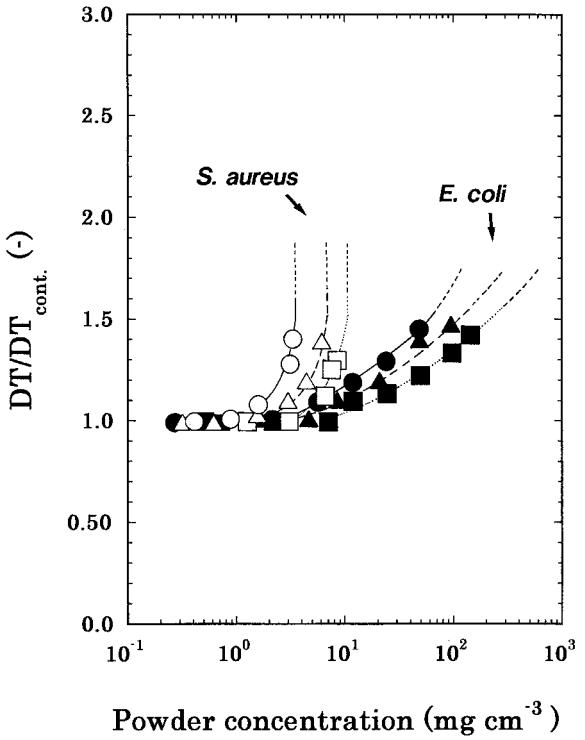

Fig. 4. The comparison in the antibacterial activity for *Staphylococcus aureus* and *Escherichia coli*. The symbols of circles, triangles and squares show the activated carbons containing ZnO obtained at 500, 700 and 900 °C, respectively.

tion peaks corresponding to ZnO of hexagonal type appeared. With an increase in the carbonization temperature, these peaks of ZnO became sharp.

Figure 2 shows an SEM-micrograph of a carbon sample carbonized at 900 °C. The shape was spherical with diameter of about 350 μm. The shapes of other samples were similar to that of a sample carbonized at 900 °C. In a detailed observation, many pores existed at the surface of the carbon sample, of which the size was found to be 10 μm and below.

The Antibacterial Characteristics of Carbon Samples. Regarding the growth of bacteria, as the electrolytes, such as organic and amino acids, are produced with the digestion of hydrocarbons and proteins in the medium, the electrical conductivity in the medium increases with bacterial growth.[12] The electrical conductivity of a growth medium, therefore, increases with an increase of the electrolytes produced, and the electrical conductivity change occurs at a bacterial concentration of about 10^7 CFU cm^{-3} in the medium.

Figure 3 shows the changes in the electrical conductivity with the incubation time of *S. aureus*, the carbon sample carbonized at 500 °C being used. In the figure, DT (detection time) shows the incubation time at which an electrical conductivity change can be detected. If a large DT value is shown by adding the carbon samples in the conductivity change, it can be judged that the carbon samples have the effect of an inhibition for bacterial growth. In the case of no addition of a carbon sample (control), the DT value was about 10h. By adding carbon samples, however, the DT value increased along with the increase in the amount of carbon powder per 1 cm^{-3} of solution (powder concentration). This result indicates that the antibacterial activity increased by increasing the concentration of activated carbon containing ZnO in the medium. Based on the changes in the electrical conductivity described above, a comparison of the antibacterial activity was carried out in all carbon samples.

In Fig. 4, a comparison of the antibacterial activity of three activated carbons is shown. The vertical axis, $DT/DT_{cont.}$, represents the ratio of the DT value at specified concentrations of carbon samples to that at no addition of carbon samples (control). If the values of $DT/DT_{cont.}$ are changed with a steep rise at the lower powder concentration, it can be judged to show stronger antibacterial activity. As the same figure, with an increase in the carbonization temperature, a pronounced change of the value in the case of *S. aureus* was found at a higher powder concentration. That is, the antibacterial activity of carbon

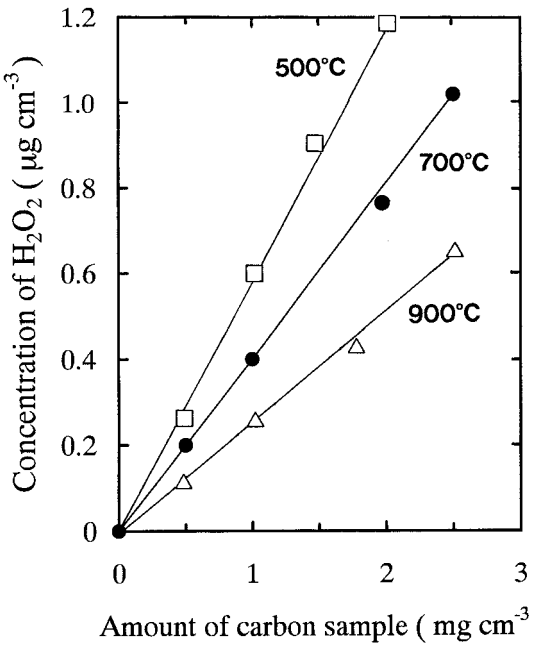

Fig. 5. The changes in the concentration of H_2O_2 with the amount of carbon samples.

samples obtained at 500 °C is clarified to be stronger than those prepared at 700 and 900 °C. The behavior in antibacterial activity of carbon samples on *E. coli* was comparable with those in the case of *S. aureus*. In *E. coli*, however, the change of the $DT/DT_{cont.}$ value occurred at a slightly higher powder concentration than that in *S. aureus*; that is, the antibacterial activity of the activated carbons containing ZnO for *E. coli* was found to be weaker than those for *S. aureus*.

The concentration of hydrogen peroxide (H_2O_2) generated from the activated carbons is shown in Fig. 5. The concentration of H_2O_2 generated increased linearly along with an increase in the powder concentration, irrespective of the carbonization temperature. In a carbon sample obtained at 500 °C, it reached a value of about 1.2 μg cm^{-3} at a powder concentration of 2.0 mg cm^{-3}, and the concentration of H_2O_2 decreased with an increase in the carbonization temperature.

Discussion

The specific surface areas of the activated carbons containing ZnO increased along with an increase in the carbonization temperature (Table 1). Nakagawa et al.[13] prepared activated carbons by carbonizing ion-exchange resins having different cations, such as zinc, nickel and copper ions. They also 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. On the present activated carbons, therefore, the increase in the specific surface areas is supposed to occur due to the pillar effect.

The amount of ZnO on the carbon sample obtained at 900 °C was about 52 wt% smaller than that in samples prepared at 500 and 700 °C (Table 1). The mass loss of ZnO is supposed 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 an increase in the activated carbons containing ZnO in the medium (Fig. 3). Also, the activity decreased along with an increase in the carbonization temperature (Fig. 4). The following three factors may affect the antibacterial activity on carbon samples containing ZnO: the pH value in the medium, zinc ion eluted from ZnO in carbon and active oxygen generated from the surface of ZnO. The pH values in physiological saline dispersing with carbon samples are about 5.6 and 5.7 (Table 1). However, the values generally do not affect the bacterial growth.[14,15] Next, the effect of zinc ion on the antibacterial activity was examined by using a physiological saline of zinc chloride with a concentration of 1.0×10^{-3} mol dm^{-3}. From the changes 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 zinc ion on the antibacterial activity was observed. By oxygen electrode analysis, H_2O_2 was detected (Fig. 5), which may contribute to the antibacterial activity, because H_2O_2 is known to be effective for antibacterial activity.[16] Therefore, the occurrence of antibacterial activity is supposed to be due to the generation of H_2O_2 from ZnO deposited in carbon samples. Based on the discussion described above, the increase in the antibacterial activity by increasing the carbonization temperature is supposed to be due to an increase in the amount of ZnO in carbon samples.

For activated carbons containing ZnO, the antibacterial activity for *S. aureus* was stronger than that for *E. coli*. In the bacteria used in this study, Sawai et al.[17] reported that the H_2O_2 which acted on *S. aureus* was stronger than that on *E. coli*. They also studied whether or not the H_2O_2 generated from ZnO was related to its antibacterial activity, by using four kinds of antibiotics.[18] In the investigation, the changes in the sensitivity of *E. coli* to the antibiotics suggested that the H_2O_2 was one of the primary factors concerning the antibacterial activity of ZnO. Saito et al.[19] reported that the H_2O_2 generated is possible to easily penetrate the cell wall of bacteria. The reason that the activated carbons containing ZnO showed a stronger antibacterial activity for *S. aureus* than that for *E. coli* is supposed to be due to the difference in the sensitivity for H_2O_2.

Finally, we note that the activated carbons containing ZnO showed the marked antibacterial activity without the presence of light. If pathological bacteria included in drinking water can be decontaminated by packing activated carbons containing ZnO into an apparatus, such as an ion-exchange column, the material is possible to use as a novel antibacterial agent to remove bacterial pollution.

Conclusion

Activated carbons containing ZnO in a highly dispersed state were prepared by carbonizing the resin-exchanged zinc ion. ZnO of hexagonal type was detected in prepared carbon samples. The specific surface area of carbon samples prepared increased along with an increase in the carbonization temperature, and the amount of ZnO in the samples decreased.

The antibacterial activity of carbon samples containing ZnO increased along with a decrease in the carbonization temperature and an increase in the amount of ZnO in the samples. 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 sa-

line dispersed with carbon samples. The occurrence of antibacterial activity was supposed to be due to the generation of hydrogen peroxide from the surface of ZnO deposited in carbon samples.

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