Preparation of ZnO Powders with Strong Antibacterial Activity under Dark Conditions

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ABSTRACT

Fine ZnO powders have been prepared from plate-like ZnO particles by hydrothermal-treatment in 3 mol/L Zn(NO₃)₂ aqueous solution at 170°C for 7 h (443 K for 25.2 × 10³ s), followed by the heating at 400~700°C for 1 h (673~973 K for 3.6 × 10³ s) in air. They show strong antimicrobial activity in the sunshade; those disinfect many kinds of bacteria even MRSA. The origin of antibacterial activity might be explained in terms of the generation of most powerful reactive oxygen species (ROS) of hydroxyl radical (OH·). As the intensity of chemiluminescence (CL) reflects the amount of ROS, the antibacterial activity has been estimated by measuring the CL emitted from the surface of ZnO in the luminol solution. Both electron spin resonance (ESR) spectroscopy and CL detection were used for identification of ROS. Furthermore, in order to increase the CL values, the ZnO powders have been ball-milled (BM) under the various conditions. It is cleared that the suitable BM conditions, such as BM time and ball size, can improve CL values greatly in comparison with those of ZnO without BM. This effect might be brought by both increasing surface area of ZnO powder and the lattice strain from “Debye effect”.

KEY WORDS
ZnO, hydrothermal treatment, antibacterial activity, under dark conditions, hydroxyl radicals

1 Introduction

Recently, bio-safe antimicrobial ZnO nanomaterials have been much focused from the viewpoints of human health because they can interact with biomolecules chemically as well as physically. It has been believed that chemical interactions of ZnO with bacterial cell lead to the photo-induced production of reactive oxygen species (ROS), formation of hydroxyl radical (OH·), or related hydrogen per oxide (H₂O₂) like TiO₂ and release of Zn²⁺ ions. In contrast, their physical interaction can reveal biocidal function through rupturing cell envelope, cellular internalization or mechanical damage. However, the mechanism of antimicrobial activity of ZnO has been still debatable. Up to now, there have been a few comprehensive reviews and many papers concerning about the preparation and mechanism of antibacterial activity of ZnO nanomaterials, such as i) (aqueous) solution route, ii) precipitation method, iii) wet chemical method, iv) hydrothermal method, and v) others: microwave, solvothermal synthesis, DC-magnetron sputter, etc. Their antimicrobial activity has been investigated from the viewpoints of particle size and surface defect structures using various kinds of Gram-positive (S. aureus and B. subtilis) and Gram-negative (E. coli and A. aerogenes) bacteria under UV radiation or in the sunshade. However, little information is available for preparation of ZnO powders hydrothermally treated in the aqueous solutions contained Zn²⁺ ions and followed by re-oxidation and ball-milling, except for our previous papers; which paper mentioned only hydrothermal treatment of ZnO at low temperatures.

Based on the idea that the antibacterial activity of ZnO is much related to reactive oxygen species (ROS), the ROS have been evaluated in terms of evaluation of luminol chemiluminescence (CL) emitted from the surface of ZnO in the dark. Thus prepared ZnO powders reveal strong antibacterial activity for E. coli, pseudomonad aeruginosa, salmonella bacteria and even an antibiotic-resistant MRSA (methicillin-resistant Staphylococcus aureus) under dark conditions at 25°C for 24 h (298 K for 86.4 × 10³ s). The present paper treats their powder morphology, crystal structure and surface conditions in relation with the generation of ROS.

2 Experimental procedure

2.1 ZnO powder preparation

As shown in Fig. 1, fine ZnO powder (ZX-100F, Sakai Chemical Industry Co., Ltd., Sakai, Osaka, Japan) with a BET surface area...
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S, of 7.83 m²/g, i.e., particle size P, of 0.137 μm, calculated from both S, and theoretical density (D,subscript;0) of 5.606 Mg/m³ (PDF #36-15451), was used as the starting material. Both this powder and a 100 mL Zn(NO₃)₂ aqueous solution with a concentration of 3 mol·L⁻¹ prepared from regent grade Zn(NO₃)₂ were put into a 150 mL PTFE (polytetrafluoroethylene) vessel in a small hydrothermal equipment. They were heated at 150~170°C for 7 h (423~443 K for 25.2 × 10³ s); these conditions were determined from our preliminary experiments. As will be described later, the powders after hydrothermal treatment (HT) were the mixtures of zinc nitrates, zinc oxide, and zinc hydroxides as shown in Table 1, differential thermal analysis and thermo gravimetry (DTA/TG), and X-ray diffraction (XRD) analysis of these compounds in air revealed that they decomposed into ZnO around 280°C (553 K), then, they were heat treated, i.e., re-oxidized into ZnO at 400~700°C for 1 h (673~973 K for 3.6 × 10³ s) in air.

Table 1  Chemical products of the hydrothermally treated ZnO powders at 170°C for 7 h (443 K for 25.2 × 10³ s) in an aqueous 3 mol·L⁻¹ (M) Zn(NO₃)₂ solution

<table>
<thead>
<tr>
<th>Group of each ZnO filling rate</th>
<th>Chemical products</th>
</tr>
</thead>
<tbody>
<tr>
<td>30~40 g-170°C/7 h (0.368 M/100 mL)</td>
<td>Zn(NO₃)₂(OH)₂ (3)</td>
</tr>
<tr>
<td>Grp. 3</td>
<td>Zn₃(OH)₄(NO₃)₂(H₂O)₂ (2)</td>
</tr>
<tr>
<td>ZnO (1)</td>
<td></td>
</tr>
<tr>
<td>20 g-170°C/7 h (0.246 M/100 mL)</td>
<td>Zn(NO₃)₂(OH)₂ (5)</td>
</tr>
<tr>
<td>Grp. 2</td>
<td>Zn₃(OH)₄(NO₃)₂ (3)</td>
</tr>
<tr>
<td>Zn₅(OH)₈(NO₃)₂(H₂O) (2)</td>
<td></td>
</tr>
<tr>
<td>ZnO (1)</td>
<td></td>
</tr>
<tr>
<td>5<del>10 g-170°C/7 h (0.064</del>0.123 M/100 mL)</td>
<td>Zn(NO₃)₂(OH)₂ (5)</td>
</tr>
<tr>
<td>Grp. 1</td>
<td>Zn₃(OH)₄(NO₃)₂ (3)</td>
</tr>
<tr>
<td>Zn₅(OH)₈(NO₃)₂(H₂O) (2)</td>
<td>ZnO (1)</td>
</tr>
</tbody>
</table>

Pulverizing of ZnO powders was performed using a planetary ball-milling apparatus (P-7, Fritsch Japan, Yokohama, Japan), zirconia 45 mL-container, and 0.3, 1.0, 2.0, 3.0 mm diameter yttria-stabilized tetragonal ZrO₂ (YTZ) balls of 50 g (5.0 × 10⁻² kg) with a powder:ball=1:10 mass ratio in 10 mL (10 × 10⁻⁶ m³) ethanol, at 6.67 rounds per sec (400 rpm, gravitational acceleration unit : 10.91 g). Furthermore, using 2.0 mm diameter YTZ balls, pulverizing was conducted for 3~180 min (1.8 × 10²~10.8 × 10³ s) under the same conditions as above.

2.2 Evaluation

As shown in left of Fig. 2, chemiluminescence (CL) of re-oxidized powders (100 μmol) in a 0.25 mL (2.5 × 10⁻⁷ m³) aqueous luminol solution (5.0 μmol/L, 5.0 × 10⁻⁹ mol·m⁻³) mixed with 3.0 mL (3.0 × 10⁻⁶ m³) carbonic acid buffer solution (NaOH/NaHCO₃: pH = 10.8) was observed in the dark condition using a CL detector (Tohoku Electronic Industrial Co., Ltd. CLD-100FC). After dropping the luminol solution in a 2 min’s (1.2 × 10² s) warming up of the detector, the intensity of CL was integrated between 1.2-6.0 × 10² s, i.e., ΣCL as shown in right of Fig. 2. X-ray diffraction (XRD, Ultima III, Smartlab, Rigaku, Tokyo, Japan) analysis using CuKα radiation with a graphite monochromator was utilized for determination of the crystalline phases and estimation of “effective Debye parameter, B eff” of ZnO powders. The latter B eff was calculated using the following equation as ln(I₀/Iₗ) = ln k – 2·B eff·(sin θ/λ)², here, ln is natural logalism, I₀ and Iₗ are the XRD intensities from each (001), (100) and (101) lattice plane of the ZnO powders after and before ball-milling, respectively, k is constant, and θ and λ are angles of XRD peaks by the radian and the wave length of CuKα of 0.15418 nm, respectively. Microstructural observation with field emission-type scanning...
electron microscopes (FE-SEM, JSM-7001FD, JSM-7800, JEOL Ltd., Tokyo, Japan) was performed on the ZnO powders prepared under various conditions. BET surface areas of powders were measured using a particle characterization analyzer (Tristar II, Micromeritics Japan, Tokyo) at room temperature. Both electron spin resonance (ESR, JES-X320, JEOL) using 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) and dimethyl sulfoxide (DMSO) based on the spin-trapping method (ST-ESR)\textsuperscript{24}, and CL with scavengers\textsuperscript{25} such as, 2-5 dimethyl furan (singlet oxygen), nitrobluetetrazolium (superoxide, ·O$_2^-$), 2-propanol (hydroxyl radical, OH·), and riboflavin (hydrogen peroxide, H$_2$O$_2$) measurements were utilized at room temperature to identify the reactive oxygen species (ROS) of ZnO powders.

3 Results and discussion

Fig. 3 shows the XRD patterns of products hydrothermally treated at 170°C for 7 h (443 K for 2.52 × 10$^3$ s) derived from ZnO powder as a function of powder filling rate in a 100 mL 3 M Zn(NO$_3$)$_2$ solution. These products were divided into 3 groups; Gr. 1 to Gr. 3 on the basis of the XRD patterns, i.e., Gr. 1, the chemical products from 5 and 10 g (5 and 10 × 10$^{-3}$ kg) ZnO powders, Gr. 2, the products from 20 g (20 × 10$^{-3}$ kg), and Gr. 3, the products from 30 and 40 g (30 and 40 × 10$^{-3}$ kg). The chemical products in each group are summarized in Table 1. In Gr. 1 there are 5 kinds of zinc compounds, however, with increasing the amount of ZnO powder the number of zinc compounds decreased to 3. This might be explained by that when the amount of ZnO is a little, Zn(NO$_3$)$_2$ is enough to react with ZnO and produce a lot of basic zinc nitrates, however, with increasing ZnO, at first Zn(OH)(NO$_3$)$_2$ is disappeared in Gr. 2 and then Zn(NO$_3$)$_3$(H$_2$O)$_2$ is not formed in Gr. 3. As these zinc nitrates salts were decomposed into ZnO around 280°C (553 K) from our preliminary experimental results of DTA/TG analysis, they were heated between 400-700°C (673-973 K). Fig. 4 displays the integrated intensity of chemiluminescence, ΣCL, of ZnO powders heated at various conditions, i.e., temperature, atmosphere such as O$_2$, air, N$_2$ or Ar. Between 400-700°C (673-973 K), ZnO powders heated in a little reductive atmosphere, especially, N$_2$/Ar at 600°C (873 K) gave the highest ΣCL values, 425 × 10$^3$ counts, suggesting that a reductive atmosphere introduced a small amount of oxygen vacancies $V'_o$ into ZnO lattice. However, the higher temperature heating than 700°C (973 K) resulted in a drop of ΣCL. This might be explained in terms of the beginning of sintering of ZnO around 700°C (973 K).

Fig. 5 (I) and (II) show the SEM photographs of ZnO powders prepared at 400°C (673 K) and 600°C (873 K) for 1 h (3.6 × 10$^3$ s) in air after the hydrothermal treatment (HT) with various reaction time from 3.5 to 21 h (12.6 to 75.6 × 10$^3$ s) are shown in Fig. 5 (I) and (II), respectively.

Analytical conditions

<table>
<thead>
<tr>
<th>Analytical conditions</th>
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<tbody>
<tr>
<td>CL reagent</td>
<td>0.25 mL luminol solution</td>
</tr>
<tr>
<td>Migration buffer</td>
<td>3.0 mL carbonic acid buffer solution (pH 10.8)</td>
</tr>
<tr>
<td>Oxidant</td>
<td>1.00 mL ZnO dispersion liquid (0.10 M/L)</td>
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</table>

Fig. 2 Method for measuring the chemiluminescence (CL) of ZnO powder under dark condition and the examples of CL curves between 120-600 s.
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These microphotographs reveal their powder morphology; i) when compared with the starting ZnO powder shown in Fig. 1, it is clear that ZnO particles have grown little from 0.137 μm (XZ-100F) to 0.1~0.2 μm (Fig. 5 [I]: 400°C/1 h (673 K/3.6 × 10^3 s) or 0.1~0.4 μm (Fig. 5 [II]: 600°C/1 h: 873 K/3.6 × 10^3 s), ii) particle size has not been varied, even though change in both the soaking time of HT and re-oxidation temperature 400°C (673 K) or 600°C (873 K). However, the powder morphology has changed from hexagonal plate-like to granular or quadrilateral particles.

In order to increase ΣCL, i.e., as will be described, to improve the antibacterial activity of ZnO, as we considered to widen the surface areas of ZnO powder, based on the idea that the disinfection might be occurred at the surface, ZnO powder was milled with a planetary ball milling (BM) apparatus with a rotating speed of 400 rpm (gravitational acceleration unit: 10.91 g) as mentioned before. Fig. 6 (i) shows the increment in BET surface area S_A as a function of milling time using 2.0 mm diameter YTZ balls (2.0 mmϕ YTZ). The values of S_A increased from 2.0 to 6.2 m^2/g up to 120 min (7.2 × 10^3 s) monotonously, however more than this the S_A value dropped a little to 6.0 m^2/g. Then ball-milled powders were observed with a FE-SEM. Representative SEM photographs of ZnO powders with the BM time 0, 60, 120, 180 min (0, 3.6, 7.2, 10.8 × 10^3 s) are shown in Fig. 6 (II); at a glance, there is a little step from 0 to 60 min (3.6 × 10^3 s), however, no big difference in morphology among 60, 120 and 180 min (3.6, 7.2, and 10.8 × 10^3 s) BM powders from SEM observation. Then, BM time dependence of ΣCL has been drawn as log-log plots. Fig. 7 (i) displays ΣCL values as a function of BM time both in logalism. It can be easily understood that after 10.0 min (6.0 × 10^3 s) BM, ΣCL value increased rapidly until 120 min (7.2 × 10^3 s) BM and dropped at 180 min (10.8 × 10^3 s). Then, to investigate the origin of ΣCL increment, the ΣCL per unit surface area (count/m^2) was calculated. If the ΣCL values depend only on the surface area, the value of ΣCL per unit area should be constant for milling time, however, Fig. 7 (ii) data suggest that BM between 30-180 min (1.8-10.8 × 10^3 s) gives the anothre effect in addition to increase in surface area. Then, XRD patterns of ZnO powders before and after BM were investigated precisely. Fig. 8 (i) and (ii) show XRD patterns of ZnO powders before and after 60 min (3.6 × 10^3 s) BM, respectively. In general, XRD pattern of ball-milled powders tend to show the low diffraction peak intensity and at the same time the wider half width, those come from pulvelized fine particles. However, when XRD patterns in Fig. 8 (i) and (ii) are compared, only the low diffraction peak intensities are recognized and the wider half width is not observed; this result indicates that only lattice strain (distortion) was introduced into ZnO powders by a planetary ball-milling using 2.0 mmϕ YTZ balls. This explanation agreed with as-mentioned SEM observation on BM powders in Fig. 6 (ii).
Then, to evaluate the structural strain (distortion), i.e., effective Debye parameter $B_{\text{eff}}$ was estimated for (001), (100) and (101) lattice planes of ZnO powder. Fig. 9 (i) indicates $B_{\text{eff}}$ behaviors for each lattice plane as a function of ball-milling time using $2.0 \text{ mm}\phi$ YTZ balls on the ZnO powder which was prepared by HT of $3 \text{ M} \text{ Zn(NO}_3\text{)}_2$ aqueous solution at $170^\circ\text{C}$ for $7 \text{ h}$ ($443 \text{ K}$ for $25.2 \times 10^3 \text{ s}$), followed by re-oxidation heat treatment of $600^\circ\text{C}$ for $1 \text{ h}$ ($873 \text{ K}$ for $3.6 \times 10^3 \text{ s}$) in air; afterward this preparation condition will be described as “standard method”. Also in Fig. 9 (ii), ΣCL values of ZnO powder as a function of ball-milling time are displayed. Good agreement in the dependence of ΣCL values on ball-milling time with that of $B_{\text{eff}}$ of (101) plane is recognized. Fig. 10 (i) and (ii) show $B_{\text{eff}}$ for each lattice plane and ΣCL values of the ZnO powder, respectively, as a function of YTZ ball size; the dependence of both $B_{\text{eff}}$ for all planes and ΣCL on YTZ ball size are similar. From these, it might be clear that $B_{\text{eff}}$ of (101) plane can reflect the dependence of ΣCL, i.e., the lattice strain (distortion) at (101) plane has much effect on the ΣCL values. This can be explained as follows;

1. Ball milling (BM) introduced ZnO powder the structural strain (distortion) in addition to the pulverizing effect; the
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5.0 g ZnO prepared under the standard method; ball-milled with 2.0mm YTZ ball 50 g, 400 rpm, Et-OH 6.3 mL

![Diagram](image)

Fig. 9  (i) Relation of effective Debye parameter $B_{eff}$ and BM time, and (ii) ΣCL values as a function of BM time.

5.0 g ZnO prepared under the standard method; milling time of 60 min ball-milled with 0.3, 1.0, 2.0 and 3.0mm YTZ balls 50 g, 400 rpm, Et-OH 6.3 mL

![Diagram](image)

Fig. 10  (i) Relation of effective Debye parameter $B_{eff}$ and diameter of YTZ balls, and (ii) integrated CL values as a function of diameter of YTZ balls.

later resulted in increase of BET surface area as mentioned in Fig. 6.

2. The former structural strain (distortion), i.e., train (distortion) can be evaluated in terms of $B_{eff}$ for each plane.

3. The dependence of $B_{eff}$ (101) on both BM time and ball size might suggest that (101) lattice plane could be easily distorted by BM because the electron clouds of two oxygen $O^{2-}$ ions with the fractional coordinates of (1/3, 2/3, 0.382) and (2/3, 1/3, 0.882) in ZnO crystal structure, spread over (101) plane partially, however, both (001) and (100) planes only contain one $O^{2-}$.

4. If it can be presumed that “z” coordinate of $O^{2-}$ might be
variable, distortion can be easily occurred in (101) plane. On the other hand, one $\text{Zn}^{2+}$ (1/3, 2/3, 1) occupies (001) plane and the electron cloud of $\text{Zn}^{2+}$ (2/3, 1/3, 1/2) spreads over (101) plane, however, no $\text{Zn}^{2+}$ occupies (001) plane.

5. Therefore, $B_{\text{eff}}$ (101) can indicate the distortion degree.

6. This distortion might enhance to form interstitial Zn atoms, which will be described later, between (101) planes of ZnO.

7. The interstitial Zn atoms, those become into $\text{Zn}^{2+}$ ions spontaneously, can produce reactive oxygen species (ROS), which brings the high ZCL values to the ball-milled ZnO powder.

To identify the ROS in thus prepared ZnO powders, electron spin resonance (ESR) using spin-trapping method (ST-ESR) was applied. Fig. 11 reveals their results; by comparing the ESR patterns shown in (i) only DMPO added and (ii) DMPO + ZnO samples, DMPO-OH signals were observed in (ii), and when DMSO was added furthermore to (ii) as shown in (iii), DMPO-OH signals was disappeared. This suggests that ROS was generated from the surface of ZnO should be hydroxyl radical (OH·) as displayed in right side of Fig. 11. Another identification of ROS had been conducted. Fig. 12 shows the CL curves of ZnO powders with scavengers such as, (i) 2-5 dimethyl furan (singlet oxygen, $^1\text{O}_2$), (ii) nitrobluetetrazolium (super oxy anion radical, superoxide, $\cdot\text{O}_2^-$), (iii) 2-propanol (hydroxyl radical, OH·), and (iv) riboflavin (hydrogen peroxide, $\text{H}_2\text{O}_2$). In Fig. 12, (a) black and (b) red curved lines represent CL data from ZnO and ZnO+scavengers, respectively. In Fig. 12 (i) and (ii), the CL values of each (b) red curved line are higher than those of (a) black lines in (iii) and (iv). These suggest that scavengers of 2-propanol and riboflavin in (iii) and (iv), reacted with hydroxyl radical OH·, and hydrogen per oxide $\text{H}_2\text{O}_2$, generated from the surface of ZnO, respectively. As hydroxyl radical OH· easily transforms into $\text{H}_2\text{O}_2$ by following equation: $2\text{OH}^· \rightarrow \text{H}_2\text{O}_2$, therefore the result of (iv) is the same as (iii). Both ESR and CL data support the generation of hydroxyl radical OH· from ZnO.

Here, antibacterial activity of thus prepared ZnO powders are considered. Fig. 13 displays the disinfect mechanism of the ZnO powders. As zinc oxide is a nonstoichiometric metal-rich compound $\text{Zn}_\delta\text{O}$, after the hydrothermal treatment at 170°C for 7 h (443 K for $25.2 \times 10^3$ s) in concentrated 3 M $\text{Zn(NO}_3)_2$ aqueous solution, followed by re-oxidation heat treatment at 600°C for 1 h (873 K for $3.6 \times 10^3$ s) in air; the value of $\delta$ might be increased, i.e., the interstitial Zn in ZnO also increased. As ZnO are not stable, they at once decomposed into $\text{Zn}^+\cdot + 2e^-$ at the ZnO surface. When water $\text{H}_2\text{O}$ in air approaches to the ZnO surface, $\text{H}_2\text{O}$ will react with $\text{Zn}^+\cdot$, then hydroxyl radical OH· will be generated by the following equation; $\text{Zn}^+\cdot + 2\text{H}_2\text{O} \rightarrow 2\text{OH}^· + 2\text{H}^- + \text{Zn}$. As mentioned before, thus prepared ZnO powders have been bio-tested based on the colony count method using various kinds of bacteria under the dark condition at 25°C for 24 h (298 K for $86.4 \times 10^3$ s). Table 2 summarizes the results; starting numbers of bacteria ($N_0$) were around $3–7 \times 10^5$, after the 24 h ($86.4 \times 10^3$ s) culture microbe, the number of each bacteria ($N_t$) has been much reduced; the rate of sterilization, i.e., $(N_t - N_o)/N_o \times 100$, are 96.9–51.1%, 99.5–99.9%, 99.998–99.995%, and 100%, for $E.\coli$, $\text{pseudomonad aeruginosa}$, $\text{salmonella bacterium}$, and MRSA.
respectively, and for the comparison, the references without the addition of ZnO were conducted. As the rate of sterilization for *E. coli* was low, then its bio-testing had been performed at another condition of 36°C (309 K), the results are shown in the lower stand of Table 2; the rate of sterilization for *E. coli* reached 100%.

As just described, the present ZnO powders proved that they showed a strong antibacterial activity even in the dark conditions.

### 4 Conclusions

Being different from the antibacterial activity of TiO$_2$ under UV, ZnO powders prepared by hydrothermal treatment of 3 M Zn(NO$_3$)$_2$ at 170°C for 7 h (443 K for 2.52 × 10$^3$ s), followed by re-oxidation treatment at 600°C for 1 h (873 K for 3.6 × 10$^3$ s) in air reveal a strong disinfect even in the shade of sun. Ball-milling has much improved the antimicrobial activity by increasing the surface...
area and introducing strain (distortion) of (101) lattice plane of ZnO. The present study proved the ZnO powders would be a high-power candidate for realizing the safe society far from catching disease, such as pseudomonad aeruginosa, salmonella bacterium, and MRSA. In future, these ZnO powders might have sterilization for influenza viruses and even bird flu virus.

Acknowledgment

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References


Table 2  The results of bio-test of antibacterial ZnO powders with various bacteria conducted at 25°C and 36°C for 24 h (298 K and 309 K for 8.64 × 10^3 s) under the dark, upper and lower, respectively.

<table>
<thead>
<tr>
<th>Reference</th>
<th>①170°C/7 h + 600°C/1 h</th>
<th>②170°C/7 h + 600°C/1 h (Ball milling 30 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>4.9 × 10^7</td>
<td>1.5 × 10^7 (*96.9%)</td>
</tr>
<tr>
<td>pseudomonad aeruginosa</td>
<td>7.0 × 10^7</td>
<td>3.5 × 10^7 (*99.5%)</td>
</tr>
<tr>
<td>salmonella bacterium</td>
<td>3.9 × 10^7</td>
<td>10 (*99.99%)</td>
</tr>
<tr>
<td>MRSA</td>
<td>2.9 × 10^7</td>
<td>&lt;10 (not detected) (*100%)</td>
</tr>
</tbody>
</table>

Testing conditions: 25°C under dark

<table>
<thead>
<tr>
<th>Reference</th>
<th>①170°C/7 h + 600°C/1 h</th>
<th>②170°C/7 h + 600°C/1 h (Ball milling 30 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>7.6 × 10^7</td>
<td>&lt;10 (not detected) (*100%)</td>
</tr>
</tbody>
</table>

Testing conditions: 36°C under dark, phosphate buffered saline *bacterial eradication rate