

## ZnO nanofluids – A potential antibacterial agent

Lingling Zhang<sup>a,\*</sup>, Yulong Ding<sup>a</sup>, Malcolm Povey<sup>b</sup>, David York<sup>c</sup>

<sup>a</sup> Institute of Particle Science & Engineering, University of Leeds, Leeds LS2 9JT, UK

<sup>b</sup> Procter Department of Food Science, University of Leeds, Leeds LS2 9JT, UK

<sup>c</sup> Procter and Gamble Newcastle Technical Centre, Newcastle-upon-Tyne NE12 9TS, UK

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### Abstract

In this work, ZnO nanofluids were produced by a medium mill with a pH value of about 7.2 and characterized by Nano-Sizer and SEM. After milling, ZnO nanofluids were formed with an average particle size of  $\sim 198.4$  nm. The ZnO nanofluids used for testing were stored for different periods (1-, 90- and 120-day) and kept in different conditions (under the light and in the dark). The antibacterial activities of these ZnO nanofluids were evaluated by estimating the reduction ratio of the bacteria treated with ZnO. The results showed that the ZnO nanofluid stored for 120 days under the light had the best antibacterial behavior against *Escherichia coli* DH5 $\alpha$ . SEM images suggest that an interaction between the ZnO particles and the *E. coli* bacteria cells caused by electrostatic forces might be a mechanism. © 2008 National Natural Science Foundation of China and Chinese Academy of Sciences. Published by Elsevier Limited and Science in China Press. All rights reserved.

**Keywords:** Antibacterial; Nanofluids; ZnO; *E. coli*; Photocatalysis

### 1. Introduction

In recent years, nanomaterials have drawn considerable interest of both academics and industrialists due to the functionalities unavailable to micron structured materials. It is found that once the materials are prepared in the ultra-fine particulate forms, significant changes could occur to their physical, chemical and electrical properties [1–3]. Anpo and coworkers reported that photocatalytic activity was increased as the size of the TiO<sub>2</sub> particles became smaller than  $\sim 10$  nm, which was resulted from the change of electronic property of TiO<sub>2</sub> particles with the reduction of the size of the particles [4]. This work concerned about the use of ZnO nanoparticles which have been shown to have some physical properties similar to those of TiO<sub>2</sub>.

In the early 1950s, scientists had already started the research on ZnO as an antibacterial material [5]. Most recently, more and more researchers have embarked on

the fundamental studies on the antibacterial activities of the metal oxides. The real move toward the use of ZnO as an antimicrobial agent was in 1995, when Sawai and his colleagues found that MgO, CaO and ZnO powders had antibacterial activities against some bacteria strains [6,7]. Many factors related to the antibacterial activities have been explored, such as the concentrations of the metal oxides particles [6–8], the particle size of the metal oxide powder [8–10] and the specific surface area of the powder [11]. These previous studies investigated the antibacterial activity of ZnO particles against *Escherichia coli*, *Salmonella typhimurium*, *Bacillus subtilis*, *Staphylococcus aureus*, etc. The main conclusions of these studies can be summarized as follows:

- (1) ZnO particles are effective for inhibiting both Gram-positive and Gram-negative bacteria. These even have antibacterial activity against spores that are high-temperature resistant and high-pressure resistant [6,7,9,12].
- (2) The antibacterial activity of MgO, CaO and ZnO appeared very near the surface, or on the surface [6].

\* Corresponding author. Tel.: +44 1133437811; fax: +44 1133432405.  
E-mail address: [prelz@leeds.ac.uk](mailto:prelz@leeds.ac.uk) (L. Zhang).

- (3) Smaller ZnO particles have a better antibacterial activity [8–10].
- (4) The antibacterial activity depends on the surface area and concentration, while the crystalline structure and particle shape have little effect. The higher the concentration is and the larger the surface area is, the better the antibacterial activity is [11].
- (5) High-temperature treatment of ZnO particles has a significant effect on their antibacterial activity. Treatment at a higher temperature leads to a lower activity [9].
- (6) The mechanisms of the antibacterial activity of ZnO particles are not well understood although Sawai et al. [12–14] proposed that the generation of hydrogen peroxide be a main factor of the antibacterial activity, while Stoimenov et al. [15] indicated that the binding of the particles on the bacteria surface due to the electrostatic forces could be a mechanism.

However, very few detailed studies were conducted on the relationship between the properties of ZnO nanofluids and their antibacterial activities. This paper will report some of our recent work on this topic. The focus will be on the characterization of the ZnO nanofluids and their antibacterial behavior under different conditions, such as the storage period for the nanofluids or the method of storing the nanofluids.

## 2. Experimental

### 2.1. Raw materials

Dry zinc oxide nanoparticles from Nanostructured and Amorphous Materials (USA), were used in this work. The primary size of the nanoparticles given by the manufacturer was 90–200 nm. Luria–Bertani (LB) medium used for growing and maintaining bacterial cultures was purchased from Sigma–Aldrich (UK). *E. coli* DH5 $\alpha$  strain used for the antibacterial tests was kindly provided by the Department of Biological Science of the University of Leeds.

### 2.2. Formulation and characterization of ZnO nanofluids

To gain more information of the shape, size distribution and morphology of the as-received nanoparticles, a scanning electron microscope (SEM) was used. Fig. 1(a) shows the SEM image of the particles obtained from the supplier. It can be seen that ZnO particles are in the form of agglomerates. Fig. 1(a) also exhibits the existence of particles that are much larger than 200 nm, and some are even micron sized.

As known, once the powder is dissolved in water, an aggregate will form, as seen in Fig. 1(a). To get an ideal ZnO nanofluid with a better size distribution, the ultrasonicator (Clifton, UK) and the Dyno-Mill (Willy A. Bachofen, Switzerland) were used. Zinc oxide nanofluid was made by dissolving zinc oxide nanoparticles in distilled

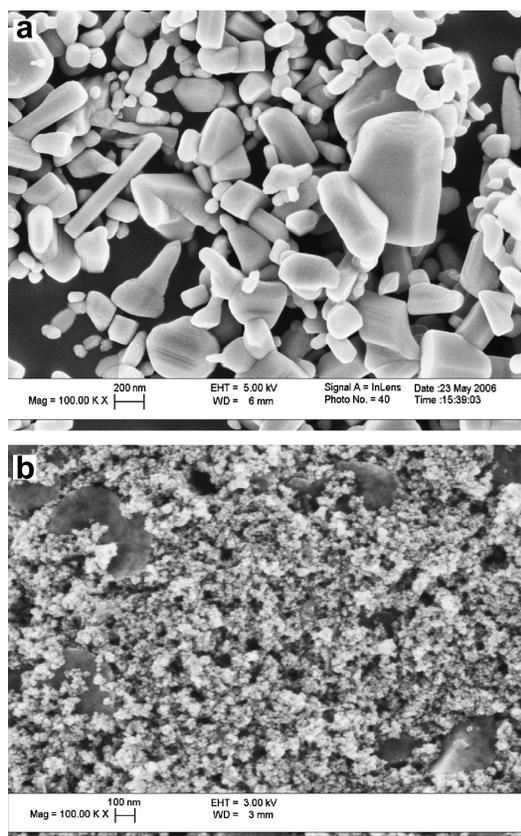


Fig. 1. SEM images of ZnO nanoparticles. (a) Before treatment; (b) after treatment.

water, sonicating for 30 min and milling for another 3 h. The pH value of the ZnO nanosuspension was adjusted to  $\sim 7.2$ . After sonication and milling, the so-called master ZnO nanofluid was produced, which had a concentration of 20 g/l. The master nanofluid prepared was then autoclaved at 121 °C for 15 min. The nanofluid was then characterized after cooling down to room temperature by using a SEM for shape, size and morphology analyses, and a Nano-Sizer (Malvern Instruments) for size distribution measurements.

### 2.3. Antibacterial tests

The numbers of the bacteria with and without the presence of different ZnO nanofluids were investigated to estimate the antibacterial activities of the produced ZnO nanofluids. All the antibacterial tests were performed in the dark. The microorganism strain used in this study was *E. coli* DH5 $\alpha$ .

For the antibacterial tests, different amounts of the master ZnO nanofluids were added to 5 ml autoclaved LB medium to get different concentrations of 10, 8, 6, 4, 2, 0.5 and 0.1 g/l. The number of the bacteria was estimated by the direct plate counting method. The test process was as follows: 50  $\mu$ l of the overnight cultured *E. coli* slurry with an approximate concentration of  $10^6$ – $10^7$  colony forming

units per milliliter (CFU/ml) was inoculated in a 5 ml LB broth medium as a blank control and in a solution mixture containing autoclaved LB broth medium and different concentrations of ZnO nanofluids. The cultures were grown at 37 °C under an agitation condition (200 rpm) for 24 h. After that, 500 µl of culture from the blank control sample and 500 µl of culture with the presence of ZnO were taken out and diluted in 10-fold increments in the LB medium. After dilution, 100 µl of the proper dilution was transferred directly onto the LB agar plate. The solution was then spread over the surface of the agar with a sterilized bent glass rod. For statistical studies, three agar plates were used for each dilution. After incubating at 37 °C for 48 h, the plates having an ideal number of colonies between 30 and 300 were counted. By knowing how much the sample was diluted prior to being plated, along with the amount of the dilution used in plating, the concentration of the viable cells per milliliter in the original sample was calculated. To be comparable, the reduction ratio of the bacteria was evaluated by the following equation [16]:  $R(\%) = \frac{A-B}{A} \times 100\%$  where  $R$  is the percentage reduction ratio,  $A$  is the number of bacterial colonies from the untreated bacteria suspension (without ZnO nanofluids) and  $B$  is the number of bacterial colonies from the bacteria culture treated by ZnO nanofluids.

The same antibacterial tests were done on the master ZnO nanofluids after the storage for 1, 90 and 120 days. On the 120th day after the ZnO nanofluid preparation, the antibacterial tests were done with the presence of the ZnO nanofluids which were stored under different conditions: one was stored under the light and the other was kept in the dark.

#### 2.4. Morphology of the bacteria

To get information on the interaction between ZnO nanoparticles and *E. coli* bacteria, 100 µl bacteria samples grown in the broth medium overnight were transferred to 10 ml of 1 g/l ZnO nanosuspension. After 5 min, one drop of the solution was taken out, dropped onto the SEM stub and air dried for the SEM investigation. After air drying, SEM sample was coated with 3 nm platinum. The sample was observed on a LEO Gemini 1530 field emission SEM at a voltage of 5 kV.

### 3. Results and discussions

#### 3.1. Characterization of ZnO suspensions (ZnO nanofluids)

Fig. 1 shows the SEM images of the ZnO nanoparticles with a magnitude of 100,000×. Fig. 1(a) is the SEM image of the ZnO particles before sample preparation, and (b) is the SEM image of the ZnO particles after ultrasonication and milling. The size of the ZnO nanoparticles given by the supplier is between 90 and 200 nm. However, the SEM image (Fig. 1(a)) suggested that the size of the original ZnO powder was obviously larger than the supplied

reading. In Fig. 1(a), some ZnO particles are of approximately 800 nm in length. It was also indicated that some ZnO particles were rod-like shaped and some irregularly shaped. After the sample preparation, the size of the ZnO particles in the suspension was dramatically dropped. Fig. 1(b) exhibits that the size of most of the ZnO particles is below 100 nm. It can be seen that the use of ultrasonication and medium milling was effective in breaking down the particles and the nanoparticle agglomerates. Both the SEM images depicted in Fig. 1 suggest that the use of Dyno-Mill reduced the particle size significantly.

ZnO particles in the ZnO suspension after milling were also analyzed using Malvern Nano-Sizer for the particle size distribution and average size (see Fig. 2). After sample preparation, the average size of the ZnO particles in the ZnO suspension was about 198.3 nm. The size distribution of the ZnO nanoparticles suggested that the ZnO particles in the nanofluids were not uniform. There were still some “large particles”, which might be the aggregates of the nanoparticles, that existed in the nanofluids. The average particle size measured by Malvern Nano-Sizer was bigger than the one found in the SEM image (Fig. 1(b)). It is because that the particle size measured by the Malvern Nano-Sizer is the hydrodynamic diameter, which is based on the Stokes–Einstein equation and expected to be larger than the actual size.

The average particle sizes of the samples with different storage periods were also tested by Malvern Nano-Sizer. The average size readings and pH values of the nanofluids

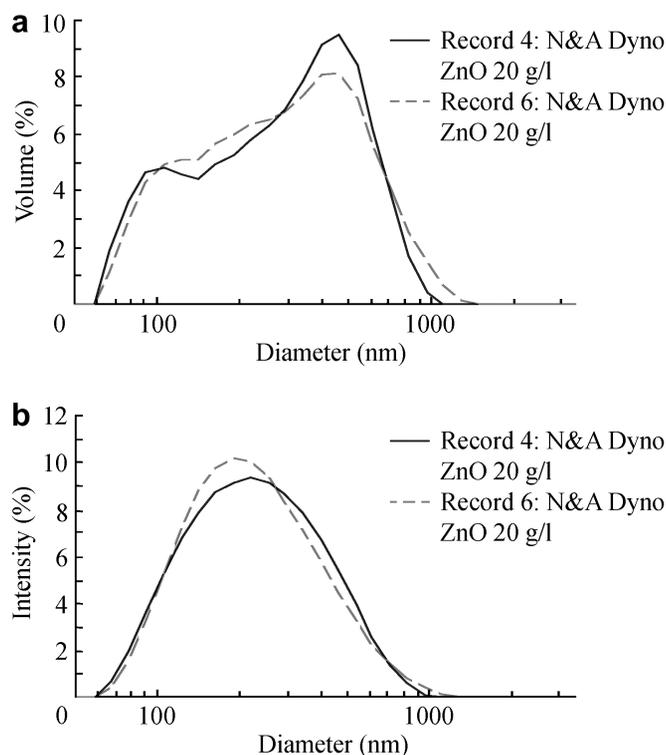


Fig. 2. Size distributions of ZnO nanoparticles by volume (a) and by intensity (b).

Table 1  
The average size of the ZnO particles

Storage time	Average size (nm)	pH value
1-day	198.4	7.2
90-day	223.1	7.2
120-day	225.9	7.2

are shown in Table 1. After the nanofluids preparation, the average size of the ZnO particles was about 198.4 nm. The average size of the ZnO particles in the nanofluids increased to approximately 223.1 nm after a 90-day storage and to around 225.9 nm after a 120-day storage. This kind of change was believed to be induced by the aggregation of the ZnO particles.

### 3.2. Antibacterial tests

Antibacterial tests were carried out on the nanofluids in various storage periods (1-, 90- and 120-day) and in different storage conditions (under the light and in the dark). The antibacterial effect of the nanofluids on the Gram-negative bacteria *E. coli* DH5 $\alpha$  is presented in Figs. 3 and 4, and Tables 2 and 3.

Fig. 3 and Table 2 show the results of the antibacterial behavior of the samples with different storage periods. The antibacterial behaviors of ZnO nanofluids against *E. coli* bacteria are exhibited. In Fig. 3, it is shown that the number of the bacterial colonies dropped with the increasing concentration of ZnO particles. For 1-day sample, the decreasing trend in the number of the bacterial colonies displayed a lag phase, where 2, 4 and 6 g/l of ZnO particles were presented in the growth medium. The decreasing trends were quite similar between 90- and 120-day samples. The number of the bacteria colonies grown in 90- and 120-day samples decreased more sharply than the one grown in 1-day sample. For 1-day sample, the presence of 10 g/l ZnO nanofluids led to 100% inhibition of the bacterial growth. The 100% inhibition concentration came to around 4 g/l in both 90- and 120-day samples. Table 2 shows the reduction ratios of the *E. coli* bacteria cells with

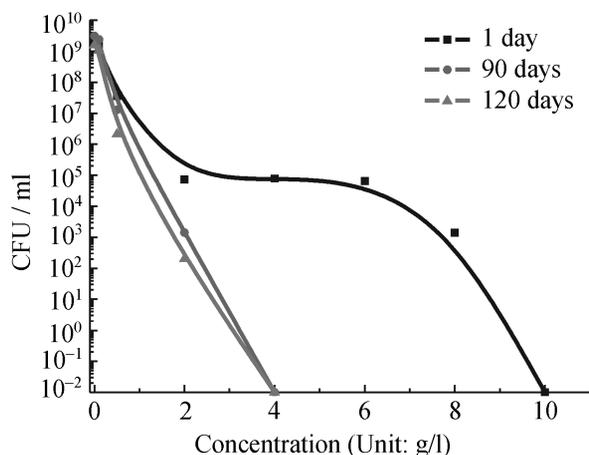


Fig. 3. Number of bacteria cells treated with ZnO samples with different storage period.

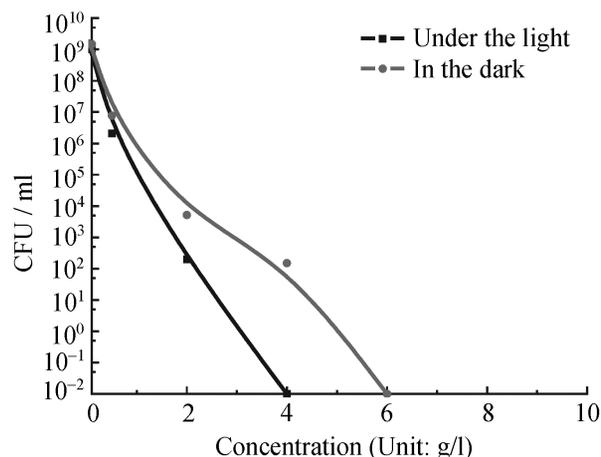


Fig. 4. Number of bacteria cells treated with ZnO samples with different storage way.

Table 2  
Reduction ratio of *E. coli* bacteria cell (%)

Sample	Concentration (g/l)						
	0.1	0.5	2	4	6	8	10
1-day	28.77	98.37	99.99	99.99	99.99	99.99	100
90-day	22.58	99.59	99.99	100	100	100	100
120-day	34.81	99.87	99.99	100	100	100	100

Table 3  
Reduction ratio of *E. coli* bacteria cell (%)

Sample	Concentration (g/l)						
	0.1	0.5	2	4	6	8	10
Under the light	34.81	99.87	99.99	100	100	100	100
In the dark	5.70	99.51	99.99	99.99	100	100	100

the presence of the ZnO nanofluids with different periods of storage. The data exhibited that treatment for 24 h with ZnO nanofluids resulted in the *E. coli* being killed by 99.99% at the concentration of 2 g/l, no matter which sample was used. However, when it came to the lower concentrations, which were 0.1 and 0.5 g/l, the reduction ratios of the bacteria cell were different with the presence of different ZnO nanofluids samples.

These results indicated that there was a higher efficiency of the antibacterial activities in the ZnO nanofluids with longer storage period. This behavior is likely due to the production of the active oxygen species, such as  $O_2^-$ , hydrogen peroxide ( $H_2O_2$ ), singlet oxygen ( $^1O_2$ ) and hydroxyl radical ( $\cdot OH$ ) [12–14]. These active oxygen species are toxic to the bacteria cell because they are very reactive and powerful oxidizing agents [14]. With longer storage period, there is more chance for the ZnO nanofluids to produce more amount of active oxygen, such as  $H_2O_2$ .

The antibacterial behavior of the ZnO nanofluids was also compared between the samples stored under the light and in the dark. The results are shown in Fig. 4 and Table 3. With

the increasing concentration of ZnO nanofluids, the number of the *E. coli* bacteria cells decreased (Fig. 4). This dropping trend of the number of bacterial cells in the sample stored under the light showed steeper than the one in the sample stored in the dark. With the presence of the same amount of ZnO nanofluids the number of bacteria cells grown in the ZnO sample in the dark was higher than that in the ZnO sample kept under the light. The data shown in Table 3 demonstrated that the reduction ratios of the *E. coli* bacteria cells in the ZnO nanofluids stored under the light were higher than the one kept in the dark at the concentrations between 0.1 and 2 g/l. When the concentration of the ZnO sample presented in the culture medium reached 2 g/l, ZnO nanofluids, both under the light and in the dark, killed 99.99% bacteria cells. With the treatment by 6 g/l ZnO nanofluids stored in the dark, there was not any bacterium in the culture medium after 24 h culture at all. This 100% killing effect happened in the ZnO sample under the light with the presence of the concentration of 4 g/l. These differences in the antibacterial activities between ZnO stored under the light and kept in the dark are presumably due to the photocatalytic reactions of the ZnO particles. When the ZnO nanofluids are stored under the light there is more chance to have certain photocatalytic reactions than the one kept in the dark. During the photocatalytic reactions, the active oxygen species which act as the oxidizing agent that causes the death of bacteria [17,18] were introduced. The production of the active oxygen species can be promoted in the ZnO sample kept under the light.

### 3.3. SEM investigation

The interaction between the bacteria cell and the ZnO particles was studied by SEM. Fig. 5 shows the SEM image of 1 g/l ZnO nanofluids in contact with *E. coli* bacteria cells. The ZnO particles used at this concentration are seen to form aggregates. The ZnO nanofluids were made at  $\sim$ pH 7, at which the zeta potential of ZnO is approximately +24 mV [8]. The overall *E. coli* surface is negatively charged at pH 7 due to the polysaccharides of lipopolysaccharide, which predominate over the amide [10]. Hence, the surface interaction of ZnO with *E. coli* is favoured mainly

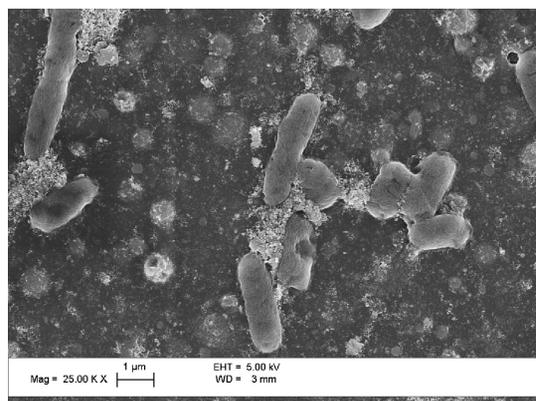


Fig. 5. SEM of bacteria and ZnO particles.

by the electrostatic forces. This suggested that part of the ZnO antibacterial activity is via contact with the bacterial membrane and the production of the active oxygen species close to the membrane.

## 4. Conclusions

The work presents the preliminary studies on the antibacterial activities of the ZnO nanofluids with different storage periods and conditions. The results have demonstrated that the ZnO nanofluids, which were stored for 120 days under the light, had the best antibacterial behavior against *E. coli* DH5 $\alpha$  bacteria. The better antibacterial activities were believed to be caused by the photocatalytic properties of ZnO particles. SEM investigation suggested that there is an interaction between the bacteria cells and the ZnO particles, which is presumably due to the electrostatic forces.

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