



Efficient disinfection of *Escherichia coli* in water by silver loaded alumina

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ABSTRACT

The catalytic inactivation of *Escherichia coli* (*E. coli*) in water by silver loaded alumina as catalyst was investigated. Ag/Al₂O₃ and AgCl/Al₂O₃ catalysts exhibited high bactericidal activity at room temperature in water with no need for any light or electrical power input. Dissolved oxygen which can be catalyzed to reactive oxygen species (ROS) was found to be essential for the strong bactericidal activities of the catalysts. Decomposition of the cell wall leading to leakage of the intracellular component and the complete lysis of the whole cell were directly observed by transmission electron microscopy (TEM). The resultant change in cell permeability was confirmed by potassium ion leakage. The different morphological changes between *E. coli* cells treated with the catalysts and Ag⁺ were also observed. The formation of ROS involved in the bactericidal process by AgCl/Al₂O₃ was confirmed by addition of catalase and ·OH scavenger. Higher temperature and pH value were found to have positive effect on the bactericidal activity of AgCl/Al₂O₃. All these results indicated that the bactericidal effect of the catalyst was a synergic action of ROS and Ag⁺, not an additive one. A possible mechanism is proposed.

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1. Introduction

It is well known that there is a worldwide shortage of fresh water resources, and infection by microorganisms is the major factor polluting drinking water. Therefore, disinfection of pathological microbes in water is very important to provide a sanitary environment and thus maintain human health [1,2]. A variety of chemical and physical techniques may be used for such disinfections. The most widely used methods in water treatment systems are chlorination, ozonation and germicidal lamps (UV) [3–5]. However, there are some disadvantages associated with each of the method. For example, potential formation of carcinogenic disinfection byproducts – chloroform and bromate, has been reported as a drawback of chlorination and ozonation.

Recently, many environmentally friendly inorganic bactericidal materials have been studied for disinfection in water and air. Generally, TiO₂ based bactericidal materials need UV light or ultrasonic irradiation to generate reactive oxygen species (ROS) [3,5]. On the other hand, silver nanoparticles and compounds containing silver are attractive because of their strong antibacterial activity, high stability, and wide antibacterial spectrum without any energy input [1,6–9]. However, so far, the mechanism underlying the bactericidal activity of silver loaded materials remains generally unclear [1,8]. Some reports have suggested that the antibacterial activity of

silver loaded materials is realized via the elution of silver ions into the system containing microorganisms, leading to cell death through cell penetration and binding at specific sites to DNA, RNA, respiratory enzymes and cellular protein [10–12]. Others attribute the bactericidal activity of silver loaded materials to catalytic oxidation involving reactive oxygen species (ROS) [1,6–8].

Our previous study has shown the inactivation efficiency of Ag/Al₂O₃ and Cu/Al₂O₃ to SARS coronavirus, bacteria and yeast in air [6]. The microbes are completely inactivated in 5 min on Ag/Al₂O₃ surface at room temperature in air. We have proposed a catalytic oxidation mechanism that ROS as bactericide in the bactericidal process are formed on the catalyst surface by activating adsorbed oxygen in air [9,13]. In this paper, we focus our attention on the bactericidal activity of Ag/Al₂O₃ in water to further identify the inactivation mechanism as a successive work to the former study [13], since the situation is very different from that in air. AgCl/Al₂O₃ was also used in this study because of its low amount of eluted Ag⁺, and thus is applicable to water resources without having adverse health effects. The objective of this study is to confirm the catalytic disinfection mechanism and primarily investigate the feasibility of this material in water.

2. Materials and methods

2.1. Preparation of the catalysts

Ag/Al₂O₃ and AgCl/Al₂O₃ (Ag 4 wt.%) were prepared by impregnation and precipitation methods, respectively, as described in our

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previous work [9,13]. The γ - Al_2O_3 powder ($200\text{ m}^2/\text{g}$) was introduced into an appropriate amount of silver nitrate aqueous solution. As for $\text{AgCl}/\text{Al}_2\text{O}_3$, an appropriate amount of ammonium chloride aqueous solution was then added into the mixture at a rate of $0.5\text{ ml}/\text{min}$, while the $\text{Ag}/\text{Al}_2\text{O}_3$ need not this step. This was followed by evaporation to dryness in a rotary evaporator at $60\text{ }^\circ\text{C}$ under reduced pressure. The resulting paste was dried at $120\text{ }^\circ\text{C}$ for 12 h, and then calcined in air at $450\text{ }^\circ\text{C}$ and $600\text{ }^\circ\text{C}$ sequentially for 3 h. Finally, the solid was ground to powder with an agate mortar, and sieved into 60 meshes.

2.2. Characterization

X-ray diffraction (XRD) was used to investigate the silver phase on γ - Al_2O_3 . In the case of 4 wt.% $\text{Ag}/\text{Al}_2\text{O}_3$, only the γ - Al_2O_3 phase was detected, but the Ag and Ag_2O phases were clearly observed at 2θ values of 33.768° , 38.128° , 44.368° and 64.468° with 8 wt.% Ag loading [14]. The absence of diffraction lines for the silver phase on 4 wt.% $\text{Ag}/\text{Al}_2\text{O}_3$ catalyst indicates that the silver is at a very high degree of dispersion [14,15]. As for 4 wt.% $\text{AgCl}/\text{Al}_2\text{O}_3$, peaks attributable to AgCl phase were observed at 2θ values of 27.76° , 32.24° , 55.86° , 57.34° and 76.54° (PDF-ICDD 85-1355, PDF-ICDD 31-1238).

Zeta-potential of the catalyst and *Escherichia coli* cells in deionized water was measured with a Zetasizer 2000 (Malvern Co., United Kingdom). Every reading of the instrument was recorded after three consistent readings were attained.

2.3. Culture of microorganisms

E. coli K 12 strain ATCC 8099 was inoculated into LB broth (Fluka Co. 61748) and grown aerobically for 24 h at $37\text{ }^\circ\text{C}$ with constant agitation. Aliquots of the culture were inoculated into fresh medium and incubated at $37\text{ }^\circ\text{C}$ for 4–5 h until reaching an exponential growth phase. Bacterial cells were collected using centrifugation at $10,000\text{ rpm}$ for 10 min, and then the pellet was washed with sterilized water. Finally, the bacterial cells were resuspended and diluted to the required cell density corresponding to 10^8 – 10^9 colony forming units per milliliter (CFU/ml) with sterilized water.

2.4. Test of bactericidal activity

An aliquot of the *E. coli* suspension was injected into sterilized water or a 0.9% saline solution in a conical flask (100 ml), then the prepared catalyst was added. The final catalyst concentration was adjusted to $50\text{ mg}/\text{L}$, and the final bacterial cell concentration was 6×10^7 or 6×10^8 CFU/ml. The reaction mixture was stirred (380 rpm) with a magnetic stirrer to prevent settling of the catalyst. All materials used in the experiments were autoclaved at $121\text{ }^\circ\text{C}$ for 20 min to ensure sterility. A bacterial suspension without any catalyst or just in the presence of Al_2O_3 host was used as a control. At certain reaction time after the addition of the catalysts, an aliquot of the reaction mixture was withdrawn and immediately diluted with 0.9% saline solution and plated on LB agar (Fluka Co. 61746) plates. The colonies were counted after incubation at $37\text{ }^\circ\text{C}$ for 24 h. All experiments were repeated three times. The bactericidal activity was measured under either aerobic or anaerobic conditions. The latter was achieved by nitrogen gas flow to examine the effect of dissolved oxygen.

Transmission electron microscopy (TEM) allows us to gain an insight into the size, structure, and morphology of the bacteria and particles. In order to avoid possible damage due to specimen preparation involving the procedures for fixing and embedding sensitive biological samples [16], native *E. coli* or the mixing suspension of treated samples was dropped onto copper grids with holey carbon film, then allowed to dry under natural conditions

and examined using a Hitachi H-7500 TEM. The same experiments were repeated three times.

2.5. Quantitative analysis of potassium and silver ions

To investigate the change in K^+ concentration during the inactivation of the *E. coli*, 1.5 ml of the treated water suspension was withdrawn and filtered through a Millipore filter (pore size $0.22\text{ }\mu\text{m}$), at each time interval, for inductively coupled plasma-atomic emission spectroscopy (ICP-AES) analysis on an Optima 2000 (Perkin-Elmer Co.). All of the solutions were prepared with Ultrapure water in this experiment. Quantitative analysis of the silver ions eluted from the catalyst in the treated suspensions was carried out in the same way. All experiments were repeated three times.

3. Results and discussion

3.1. Bactericidal activity of silver loaded alumina on *E. coli*

The bactericidal activity of silver loaded alumina in deionized water at room temperature was investigated. Fig. 1b, d and e show the survival changes of *E. coli* in the presence of different catalysts as a function of time. Under the control condition, the viable cell numbers were counted without the addition of any catalyst (shown in Fig. 1a). It was clearly observed that in the presence of $\text{Ag}/\text{Al}_2\text{O}_3$ and $\text{AgCl}/\text{Al}_2\text{O}_3$ the *E. coli* in the suspension was completely inactivated after only 30 min and 60 min, respectively. Silver supported alumina catalysts were so effective that the surviving *E. coli* cells decreased sharply even in the first 10 min. However, with Al_2O_3 addition, the viable cell count was similar to that in the control even after 60 min. This means that the adsorption of *E. coli* on alumina is negligible, and the dramatic decrease in *E. coli* survival is ascribed to the highly efficient bactericidal activity of $\text{Ag}/\text{Al}_2\text{O}_3$ and $\text{AgCl}/\text{Al}_2\text{O}_3$.

It is well known that Ag^+ at high concentrations exhibits bactericidal activity [17–19], and Ag^+ eluting from $\text{Ag}/\text{Al}_2\text{O}_3$ cannot be avoided under the experimental conditions. To decrease the amount of eluted Ag^+ , $\text{AgCl}/\text{Al}_2\text{O}_3$ was used in this study. The quantitative analysis of eluted silver ions by ICP-AES showed that there was $0.897\text{ mg}/\text{L}$ Ag^+ and $0.081\text{ mg}/\text{L}$ Ag^+ eluted from $\text{Ag}/\text{Al}_2\text{O}_3$ and $\text{AgCl}/\text{Al}_2\text{O}_3$, respectively, when the catalysts were immersed singly in deionized water in the absence of *E. coli* for 60 min. As shown in

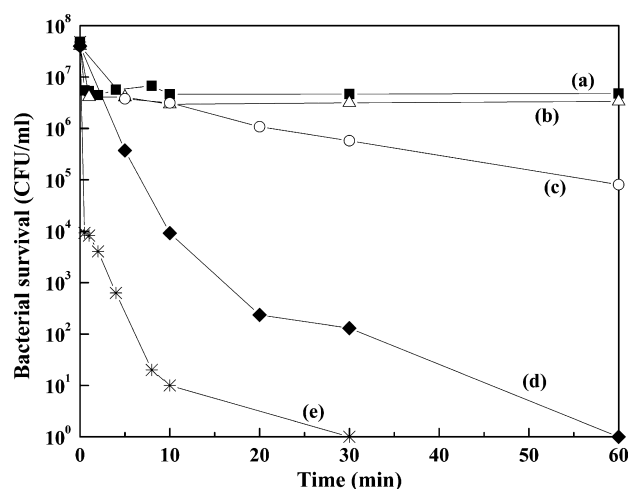


Fig. 1. Bactericidal activity in deionized water at room temperature: (a) control, (b) $50\text{ mg}/\text{L}$ Al_2O_3 , (c) $1\text{ mg}/\text{L}$ Ag^+ (d) $50\text{ mg}/\text{L}$ $\text{AgCl}/\text{Al}_2\text{O}_3$ and (e) $50\text{ mg}/\text{L}$ $\text{Ag}/\text{Al}_2\text{O}_3$. Initial bacterial concentration: 6×10^7 CFU/ml.

Fig. 1d and e, $\text{Ag}/\text{Al}_2\text{O}_3$ with the larger amount of Ag^+ actually exhibited stronger bactericidal activity than $\text{AgCl}/\text{Al}_2\text{O}_3$, which implied that Ag^+ may play an important role in the bactericidal process. However, the bactericidal activity of 1 mg/L Ag^+ (shown in Fig. 1c) was much lower than either of the catalysts, indicating that the effect of Ag^+ alone cannot completely explain the bactericidal activity.

3.2. Transmission electron microscopy imaging and K^+ leakage

TEM was used to directly observe the morphological changes in the bacterial cells after being treated with the catalysts or Ag^+ in deionized water and/or physiological saline. Fig. 2a shows a TEM image of the untreated *E. coli* cells. It is clear that the cells show uniform electron density, suggesting that they are in a normal condition without environmental disturbance.

As shown in Fig. 2b, slight morphological changes occurred in the *E. coli* cells after Ag^+ treatment for 3 h. The cytoplasmic membrane shrank and became detached from the cell wall (arrow in Fig. 2b). However, no discernible degradation in the membrane structures of *E. coli* cells was observed with the 3 h treatment by Ag^+ , and they maintained an intact configuration compared with the normal cells shown in Fig. 2a. The same phenomenon was observed by Yamanaka et al. [16], and they proposed that the Ag^+ penetrated into the interior of the cells through ion channels without causing damage to the cell membranes. Feng et al. [20] also reported that Ag^+ ions were detected inside the *E. coli* cells by energy dispersive spectroscopy (EDS), indicating the interaction with thiol groups in cytoplasmic protein leading to the inactivation of the bacterial proteins.

In contrast, the *E. coli* cells treated with $\text{Ag}/\text{Al}_2\text{O}_3$ and $\text{AgCl}/\text{Al}_2\text{O}_3$ under the same conditions showed dramatic morphological changes during the process of cell death. In physiological saline, the cells swelled to a much larger size than the untreated ones, and the cell envelope of *E. coli* was significantly damaged from

the top of the cell (shown in Fig. 2c and d), resulting in leakage of the intracellular ingredients. This phenomenon was similar to that of *E. coli* cells damaged by H_2O_2 , as reported by Shin et al. [21]. For the cells treated in deionized water (shown in Fig. 2e and f), the whole cells completely disappeared, and nothing was observed except for a large number of fibriform cell pieces. Furthermore, some electron-dense granules adherent to the fibriform pieces can be clearly observed (arrows in Fig. 2e and f). As reported by Feng et al. [20], some of the stimulated proteins produced by the cells would agglomerate after the attack by silver ions, surrounding the nuclear region. Further investigation indicated that the DNA molecules became condensed like a twisted string losing their replication abilities. According to these conclusions, the fibriform cell pieces observed in Fig. 2e and f were possibly due to lysis of the bacterial cells leading to leakage of damaged DNA molecules [22]. This implies that a stronger oxidizing intermediate was formed to destroy the cells more thoroughly.

It is well known that the outer membrane plays an essential role in providing a barrier of selective permeability for *E. coli* and other Gram-negative bacteria [23]. On the basis of the TEM investigation, after treatment with the catalysts, the cell wall and cell membrane would be destroyed, subsequently leading to a change in cell membrane permeability and leakage of intracellular substances. K^+ exists universally in bacteria [24–26], and plays an important role in the regulation of polysome content and protein synthesis. Therefore, K^+ leakage from *E. coli* was used in this work to further examine the permeability of the cell membrane. Fig. 3a shows the dependence of K^+ concentration as a function of contact time with $\text{AgCl}/\text{Al}_2\text{O}_3$ catalyst. Native *E. coli* cells of the same concentration were also investigated as a control (shown in Fig. 3b).

In the case of the control experiment, without addition of any catalyst, there was almost no K^+ leakage from *E. coli* cells (some cells may be damaged during the operation), and the amount of K^+ was very steady in 120 min. However, in the presence of $\text{AgCl}/\text{Al}_2\text{O}_3$, K^+ would leak out from the bacterial cells just 5 min after

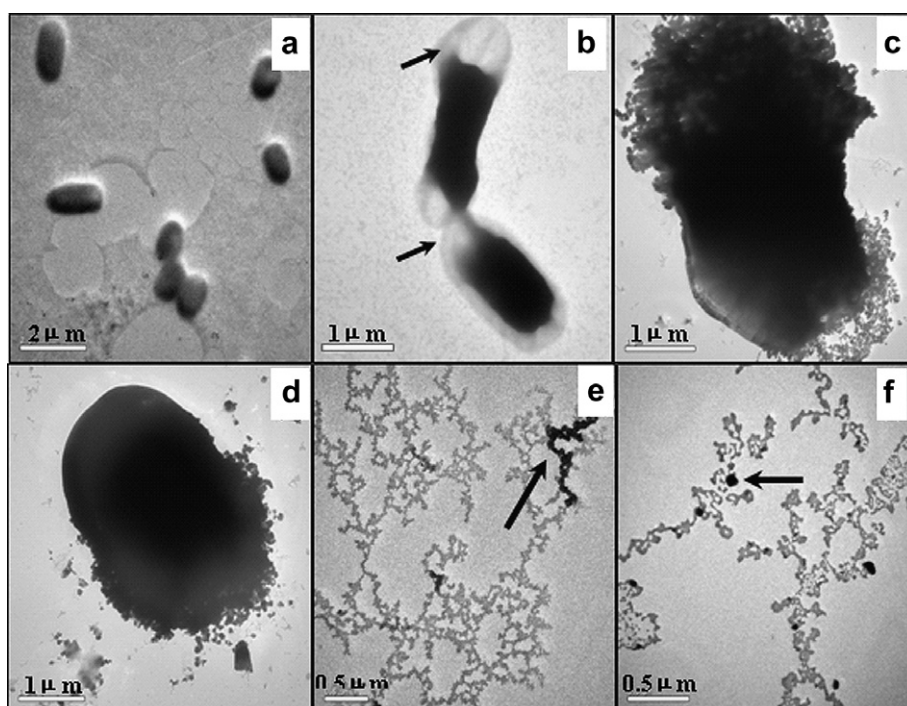


Fig. 2. TEM images of (a) untreated *E. coli* cells ($\times 15,000$) and treated samples after treatment with (b) 1 mg/L Ag^+ ($\times 30,000$), (c) $\text{Ag}/\text{Al}_2\text{O}_3$ in physiological saline ($\times 30,000$), (d) $\text{AgCl}/\text{Al}_2\text{O}_3$ in physiological saline ($\times 30,000$), (e) $\text{Ag}/\text{Al}_2\text{O}_3$ in deionized water ($\times 60,000$) and (f) $\text{AgCl}/\text{Al}_2\text{O}_3$ in deionized water ($\times 60,000$) for 3 h at room temperature. Catalyst concentration: 50 mg/L.

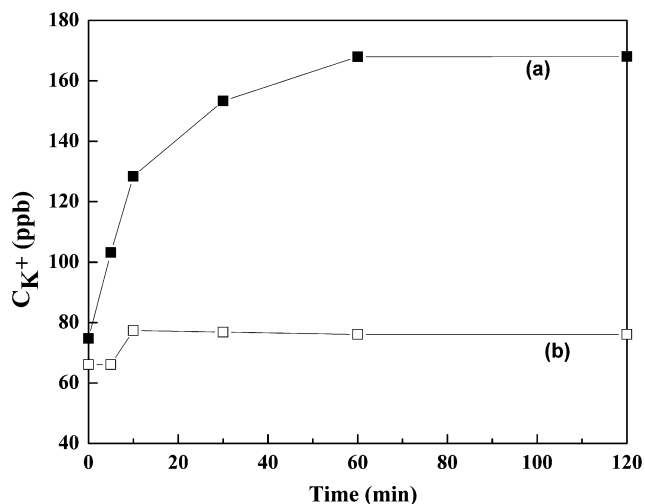


Fig. 3. Leakage of K^+ from *E. coli* cells in the presence (a) and absence (b) of 50 mg/L $AgCl/Al_2O_3$ as a function of time at room temperature.

addition of the catalyst. The K^+ concentration increased remarkably with increasing time, which should be attributed to a notable change in the structure of the cell wall (see Fig. 2f). The K^+ level reached relatively steady values after 60 min, when 100% cell damage was achieved (see Fig. 1d). The consistency of K^+ leakage with the bactericidal efficiency confirmed the decomposition of the cell wall and the cell membrane, which was supposed to be achieved by the catalytic oxidation of ROS with strongly oxidizing properties.

3.3. Effect of dissolved oxygen

Since oxygen is reported essential for the formation of ROS on the catalyst in air [6,9], the bactericidal activity under both aerobic and anaerobic conditions obtained by oxygen or nitrogen bubbling was investigated. All the experiments were performed in physiological saline, in order to exclude the effect of Ag^+ (for a large amount of Cl^-) [7].

Fig. 4 shows the effect of nitrogen and oxygen gas bubbling on the bactericidal activity of the catalyst in physiological saline at room temperature. The bubbling was continued throughout the

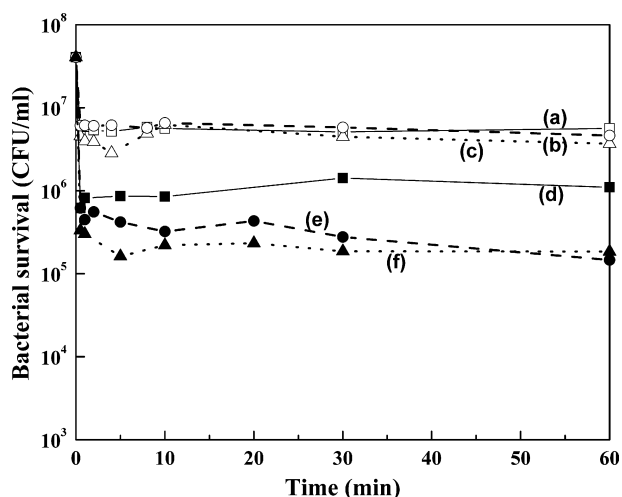


Fig. 4. Effect of oxygen on the bactericidal activity in physiological saline at room temperature: (a) $Al_2O_3 + N_2$, (b) $AgCl/Al_2O_3 + N_2$, (c) $Ag/Al_2O_3 + N_2$, (d) $Al_2O_3 + O_2$, (e) $AgCl/Al_2O_3 + O_2$ and (f) $Ag/Al_2O_3 + O_2$. Catalyst concentration: 50 mg/L; initial bacterial concentration: 6×10^7 CFU/ml.

treatment period. Clearly, the inactivation of *E. coli* by silver loaded alumina under aerobic conditions (Fig. 4e and f) was more efficient than that under anaerobic conditions (Fig. 4b and c). This suggested that ROS generated on the catalyst surface is an essential factor for the expression of the bactericidal activity in the presence of catalysts [1,8]. The same conclusion was reached in deionized water by our previous work [6,9]. However, without the presence of Ag^+ , the bactericidal activity decreased in physiological saline even under aerobic conditions, compared with that in deionized water (Fig. 1d and e). Furthermore, the bactericidal activities of Ag/Al_2O_3 and $AgCl/Al_2O_3$ did not show a sharp difference and were still stronger than that of the Al_2O_3 host. All of these results indicated that the fixed silver species and/or Ag^+ play an important role in the process of activating O_2 to ROS.

Indeed, silver loaded catalysts have been reported to produce H_2O_2 in the presence of oxygen species [8,17,19]. As mentioned above, the effect of Ag^+ on bacteria could be excluded in physiological saline, and the bactericidal effect was supposed to be attributed to the catalytic oxidation of H_2O_2 , since the morphological changes of bacteria cells (see Fig. 2c and d) are identical to those damaged by H_2O_2 solution [21].

Catalase is a well-known scavenger for H_2O_2 [1], therefore catalase was introduced in the bactericidal experiment to investigate whether H_2O_2 was formed in this process. As can be seen from Fig. 5, with the addition of 286 units/ml catalase the bactericidal activity of the catalyst was inhibited drastically, while as shown in the blank experiment, the same concentration of catalase itself has no effect on the bacterial survival. This result indicates that the formation of H_2O_2 play an important role during the bactericidal process. The addition of catalase had two effects [1]: one was transformation of H_2O_2 to water, the other was interception of the route of formation of $\cdot OH$, because $\cdot OH$ was formed by the decomposition of H_2O_2 . As have been reported [1,8], the transitional metals, such as silver and ferric ions could catalyze the oxidizing action of H_2O_2 by forming $\cdot OH$ and/or $\cdot O_2^-$, through a so-called Fenton-like reaction. As a result, $\cdot OH$ and other ROS such as $\cdot O_2^-$ and H_2O_2 , simultaneously contribute to the efficient inactivation of *E. coli* cells.

Furthermore, among ROS, H_2O_2 is reported to present a low rate of decomposition in water ensuring long lasting residual bactericidal effects [27]. However, silver ions could improve the oxidizing action of H_2O_2 by forming $\cdot OH$ known as the strongest oxidant

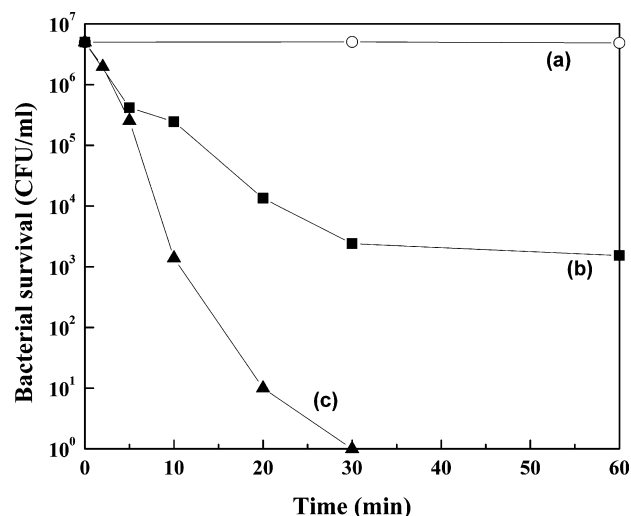


Fig. 5. Effect of catalase against H_2O_2 on the bactericidal activity of Ag/Al_2O_3 at room temperature: (a) 286 units/ml catalase only; (b) 286 units/ml catalase + 50 mg/L $AgCl/Al_2O_3$ and (c) 50 mg/L $AgCl/Al_2O_3$ only. Initial bacterial concentration: 7×10^6 CFU/ml.

[28,29] as mentioned above, leading to lysis of the whole bacterial cells (see Fig. 2e and f). This may be the reason why the time required for complete inactivation by the catalyst in physiological saline was much longer than that in deionized water. Since the combined use of H_2O_2 and Ag^+ exhibited improved bactericidal effects compared to H_2O_2 and Ag^+ alone [27–29], the simultaneous presence of H_2O_2 and Ag^+ in deionized water could adequately explain the much higher bactericidal activity compared with that in physiological saline. We supposed that H_2O_2 was first formed on the catalyst surface by activating adsorbed O_2 , then H_2O_2 was further catalyzed to a stronger ROS ($\cdot\text{OH}$, etc.) by eluted Ag^+ . Since the quantitative analysis of the generated ROS is complex, an indirect method by $\cdot\text{OH}$ scavenger was used to prove the production of ROS.

3.4. Effect of $\cdot\text{OH}$ scavenger

An excess of methanol is well known to act as an efficient scavenger of $\cdot\text{OH}$ [30–32]. Fig. 6 shows the effect of methanol on the inactivation of *E. coli*. Methanol with no catalyst showed very little bactericidal effect on *E. coli* under the experimental conditions (curve a), indicating that methanol itself at the tested concentration is not toxic to *E. coli*. The rate of inactivation of *E. coli* by $\text{AgCl}/\text{Al}_2\text{O}_3$ was inhibited with the addition of methanol as shown in Fig. 6b–d, and this result indicated that *E. coli* cells were inactivated by direct oxidation of $\cdot\text{OH}$. Increases in the methanol concentration increased the competition for the oxidizing species, decreased the inactivation rate of *E. coli*, strongly supporting the view that $\cdot\text{OH}$ is one of the fatal factors for the bactericidal activity of silver loaded alumina catalysts in water.

Since the inactivation was not totally inhibited, other ROS species concurrently generated along with $\cdot\text{OH}$, such as H_2O_2 , O_3 , and $\cdot\text{O}_2^-$ [33], are involved during the bactericidal process. In our previous work, superoxide dismutase (SOD) was used as the scavenger of $\cdot\text{O}_2^-$ under the same conditions, and the bactericidal activity decreased with increasing addition of SOD to deionized water [13]. In addition, methanol does not react with $\cdot\text{O}_2^-$, suggesting that $\cdot\text{OH}$ is not the sole oxidant responsible for the inactivation of *E. coli*. Further investigation is required to elucidate other possible roles.

3.5. Effect of temperature

It is well known that the growth of the bacteria is significantly affected by temperature, and grows much more rapidly at higher

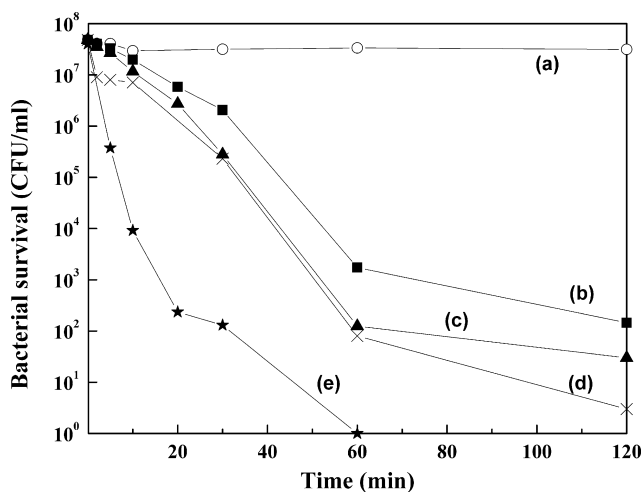


Fig. 6. Survival changes of *E. coli* with 50 mg/L $\text{AgCl}/\text{Al}_2\text{O}_3$ under different conditions: (a) 0.3 M MeOH with no catalyst, (b) 0.3 M MeOH, (c) 0.2 M MeOH, (d) 0.1 M MeOH and (e) only $\text{AgCl}/\text{Al}_2\text{O}_3$ in deionized water with initial bacterial concentration of 6×10^7 CFU/ml.

temperatures within a certain temperature range. In practice, the catalysts would be used at various temperatures in inhabited environment conditions, so the effect of temperature on the bactericidal activity of $\text{AgCl}/\text{Al}_2\text{O}_3$ against *E. coli* was investigated. Because the optimum temperature for the growth and reproduction of *E. coli* is 0–42 °C, the temperatures selected in this study were 25, 35, and 40 °C, respectively, in accordance with normal temperatures in the human living environment. The reaction temperatures were exactly controlled by a thermostatted water bath with magnetic stirrer.

The curves in Fig. 7 demonstrated dependence of bacteria survival on the contact time at various temperatures in the presence of $\text{AgCl}/\text{Al}_2\text{O}_3$. It can be seen that the bactericidal activity of the catalyst increased dramatically with temperature increase, especially at 40 °C, where the bactericidal activity was so effective that the *E. coli* cells were completely inactivated in only 5 min. This result confirmed that a catalytic oxidation which is closely related to temperature was involved during the bactericidal process. Since $\text{AgCl}/\text{Al}_2\text{O}_3$ exhibited stronger bactericidal activity at higher temperature, it is a promising material to disinfect bacteria efficiently at various temperatures under living conditions.

3.6. Effect of pH value

pH value is another factor which may play an important role during the bactericidal process in water. Therefore, the effect of pH on the bactericidal activity of $\text{AgCl}/\text{Al}_2\text{O}_3$ was investigated at room temperature in deionized water. Because the proper pH for normal growth of *E. coli* ranged from 4.0 to 9.0, the pH value was adjusted to about 4.0 and 8.0, respectively by addition of HNO_3 or NaOH to the catalyst suspension before injecting the bacterial suspension. pH 6.0 is the initial pH value of deionized water without any adjustment. In order to dissociate the effect of the pH modification on bacteria from the specific impact of the bactericidal process by $\text{AgCl}/\text{Al}_2\text{O}_3$, HNO_3 or NaOH was directly added to the bacterial suspension in the absence of $\text{AgCl}/\text{Al}_2\text{O}_3$, adjusting the pH value to the similar range as the control experiments.

As can be seen from Fig. 8a, the growth of *E. coli* was independent on the initial value of pH between 4.0 and 8.0 in our control experimental conditions. However, the bactericidal activity increased obviously with pH increasing in the presence of $\text{AgCl}/\text{Al}_2\text{O}_3$. Furthermore, the bactericidal activity did not increase obviously from 6.0 to 8.0 of the pH value. Since the charges of the catalyst and bacteria are both closely related to pH value, zeta

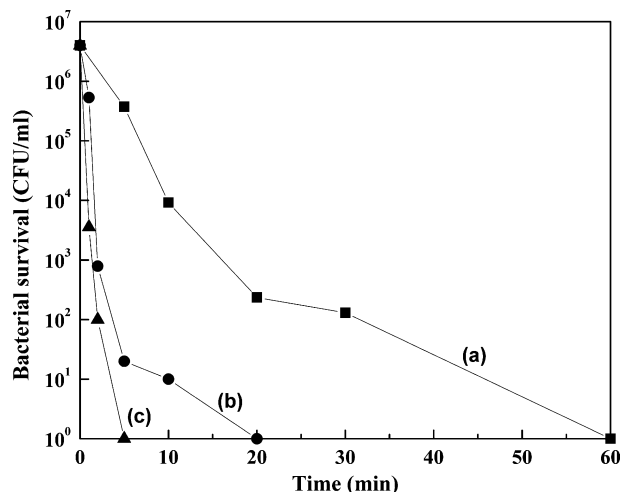


Fig. 7. Effect of temperature on the bactericidal activity of 50 mg/L $\text{AgCl}/\text{Al}_2\text{O}_3$: (a) 25 °C; (b) 35 °C and (c) 40 °C. Initial bacterial concentration: 7×10^6 CFU/ml.

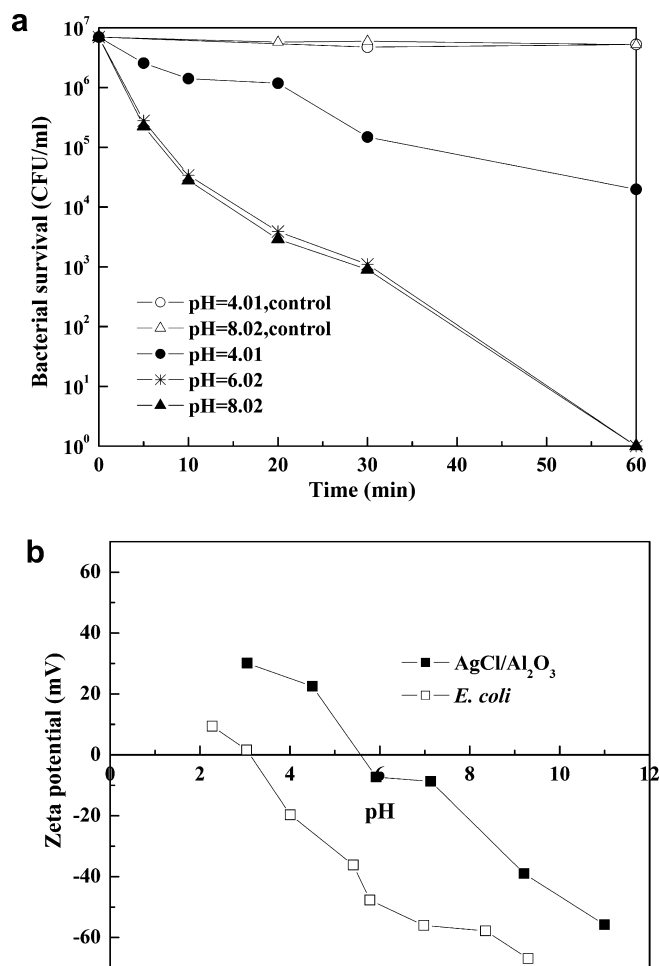


Fig. 8. (a) Effect of pH value on the bactericidal activity of 50 mg/L AgCl/Al₂O₃ with initial bacterial concentration of 7×10^6 CFU/ml; (b) Zeta potentials for suspensions of AgCl/Al₂O₃ and *E. coli* in deionized water at various pH values.

potentials of the bacteria and AgCl/Al₂O₃ were determined at various pH values as shown in Fig. 8b. Though the surface of the bacteria was positively charged and the surface of the catalyst was negatively charged in the range of pH 3.0–5.5, the bactericidal activity of the catalyst was quite low in acidic condition as shown in Fig. 8a. On the other hand, in the range of pH 5.5–12, the surface of the bacteria and catalyst were both negatively charged, whereas the bactericidal activity of the catalyst was high when pH > 6.0 as shown in Fig. 8a. Therefore, the electrostatic attraction between *E. coli* and the catalyst did not play an important role in the bactericidal process. In other words, the effect of *E. coli* adsorption onto the catalyst surface on the inactivation of *E. coli* was neglectable in our experimental condition with pH 6.0, which indicated that a catalytic oxidation was involved during the bactericidal process.

Recently, the formation and decomposition of H₂O₂ in an aqueous TiO₂ dispersion under visible light irradiation have been reported [34,35], the generation of active ·OH radicals were also observed in the decomposition of H₂O₂ which possess strong oxidation ability and may cause oxidation reaction of organic compounds adsorbed on the TiO₂ surface. The higher the pH value was, the more rapid decomposition of H₂O₂ was, and the stronger the catalytic oxidation ability was. All these results could well explain the higher bactericidal activity of AgCl/Al₂O₃ at higher pH value, since the involvement of H₂O₂ has also been observed in our experiment as mentioned above (see Fig. 5). However, when the

pH value was too high, the formation of H₂O₂ ($pK_a = 11.56$) was blocked, and the catalytic oxidation ability would not increase any more [35]. It solidly confirmed the catalytic oxidation mechanism mediated by different kind of ROS. Furthermore, the high bactericidal activity in neutral condition suggested that this catalyst is a very potential material to apply in drinking water disinfection.

Ag⁺ ions do elute from the catalyst surface, but the amount is no more than 0.09 mg/L in 1 h, and the World Health Organization (WHO) declared that silver does not cause adverse health effects and set a secondary maximum concentration level (MCL) of 90 µg/L [19,27,36]. Therefore, silver loaded on alumina was thought not only to increase the effectiveness of this catalyst system but also to be safe and economical, since the catalyst could be used several times after regeneration.

4. Conclusion

This study demonstrated an efficient bactericidal catalyst with strong catalytic inactivation of *E. coli* at room temperature in water. The fixed silver species and/or eluted Ag⁺ strongly enhanced the bactericidal activity of the catalyst by catalyzing adsorbed O₂ to ROS, such as H₂O₂, ·OH and ·O₂⁻. ROS with strongly oxidizing properties resulted in destruction of the cell wall of the bacteria and finally the complete lysis of the bacterial cell. The catalytically bactericidal effect should be considered as a synergic action of ROS and Ag⁺, not as an additive one. Since the bactericidal activity of the catalyst increased with temperature increasing, and showed good performance at neutral pH value condition in water, it may provide a novel approach for the design of other bactericidal materials applied in drinking water disinfection. Further study and direct proof of ROS are needed to clarify the details of these actions.

5. Abbreviations

ROS	reactive oxygen species
TEM	transmission electron microscopy
SARS	severe acute respiratory syndrome
XRD	X-ray diffraction
LB	lactose broth
CFU/ml	colony forming units per milliliter
SOD	superoxide dismutase
ICP-AES	inductively coupled plasma-atomic emission spectroscopy

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