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Comparative study of visible-light-driven photocatalytic inactivation of two different wastewater bacteria by natural sphalerite

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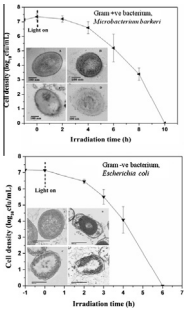
HIGHLIGHTS

- Natural sphalerite can inactivate both Gram +ve and Gram −ve under FT irradiation.
- Major ROS(s) for inactivation affected by the structure of bacterial cell envelope.
- Gram −ve bacteria is easier to be inactivated than Gram +ve bacteria.
- Inactivation of the Gram +ve and Gram −ve bacteria starts from cell envelope.

GRAPHICAL ABSTRACT



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ABSTRACT

The photocatalytic inactivation of two different wastewater bacteria, Gram-positive (+ve) bacterium *Microbacterium barkeri* and Gram-negative (−ve) bacterium *Escherichia coli*, were comparably investigated with natural sphalerite (NS) as a novel low-cost photocatalyst. The natural sphalerite was able to inactivate 10^5 cfu/mL of the Gram-positive (+ve) bacterium *M. barkeri* within 10 h at neutral pH while 10^7 cfu/mL of the Gram-negative (−ve) bacterium *E. coli* was inactivated within 6 h. In addition, the pH effect on the photocatalytic inactivation of bacteria was studied, and the results suggested that the amount of H_2O_2 produced under different pH influenced the bacterial inactivation efficiency. The major reactive species for photocatalytic inactivation by the NS were determined using multiple scavengers, and H_2O_2 was found to be the major ROS in *M. barkeri* inactivation, while both H_2O_2 and e^- contributed to *E. coli* inactivation. Different mechanism between Gram-positive (+ve) and Gram-negative (−ve) bacteria was mainly due to the different thickness of the cell wall, as indicated by direct observation of the cell wall and cell membrane by transmission electron microscopy and leakage detection of potassium ions.

1. Introduction

Since Matsunaga et al. [1] first reported that TiO_2–UV photocatalysis could successfully inactivate bacterial cells in water, photocatalytic technology has been extensively studied and proven to be an efficient, safe, and promising alternative for microbial inactivation. However, TiO_2 can only be excited by UV, which covers only 4% of the solar spectrum. Hence, it is crucial to develop new photocatalysts that can be excited by visible light (VL). Although many synthetic photocatalysts show good inactivation efficiencies under VL [2–4], the production of synthetic visible-

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light-driven (VLD) photocatalysts is still in small quality and cannot satisfy large-scale applications in wastewater treatment. Obviously, the acquisition of large quantities of low-cost VLD photocatalysts is urgent in need.

Natural minerals may be an alternative to many synthetic photocatalysts for large-scale applications in wastewater treatment because they can be available in large quantities from the Earth's surface. For example sphalerite has billion tons mineral deposits on the Earth. However, with a bandgap of 3.6 eV, the application of pure sphalerite (ZnS) in photocatalysis is very difficult due to its low photocatalytic efficiency under VL [5]. Fortunately, natural sphalerite (NS), collected from Huangshaping deposit in Hunan Province, China, containing other metal ions such as Fe^{2+} and Cd^{2+} in which modifies the band structure of ZnS, was found as a novel low-cost photocatalyst [6–8]. The bandgap of NS is only 2.95 eV [8]. Most importantly, the NS has an outstanding advantage over other synthetic VLD photocatalysts as a large amount of NS can be obtained from mining sites at comparatively low cost. We believe that the NS has promising applications in wastewater disinfection if the photocatalytic inactivation mechanism of different bacteria can be well revealed.

Recently, controversial conclusions have been drawn from the photocatalytic inactivation of different kind bacteria, such as Gram positive (+ve) and Gram negative (−ve) bacteria and the photocatalytic inactivation mechanism of different bacteria was still not clear. Čík et al. [9] report that the photocatalytic inactivation of Gram −ve bacterium *Escherichia coli* (*E. coli*) was more efficient than that of the Gram +ve bacterium *Staphylococcus aureus* (*S. aureus).* And Pal et al. [10] found that two strains of *E. coli* (Gram −ve bacteria) were more effectively inactivated than four strains of *Bacillus subtilis* (*B. subtilis*) (Gram +ve bacteria) . But van Grieken et al. [11] reported that the Gram −ve bacterium *E. coli* and Gram +ve bacterium *Enterococcus faecalis* (*E. faecalis*) had no significant difference in both TiO_2 suspension and immobilized TiO_2 photocatalytic systems. To date, there is no study comparing the photocatalytic inactivation of Gram −ve with Gram +ve bacteria to reveal the role of ROSs by using natural minerals photocatalyst. Hence, it is important to investigate the efficiency and potential mechanism of photocatalytic inactivation towards different bacteria by NS under VL irradiation.

Thus, in this study, different responses of two different wastewater bacteria to VL driven photocatalytic inactivation by NS photocatalyst were mainly compared. The investigation of photocatalytic inactivation mechanism were also attempted, and the applicability of NS towards two wastewater bacteria was tested employed a Gram +ve bacterium, *Microbacterium barkeri* (*M. barkeri*), and a Gram −ve bacterium, *E. coli*, isolated from the sludge samples collected from the Shatin Sewage Treatment Works in Hong Kong.

2. Materials and methods

2.1. Materials

The natural sphalerite (NS) used in this work was collected from the Huangshaping deposit in Hunan Province, China, and mechanically crushed and milled at the mining site. All NS particles were ground into powder and then sieved through 340-mesh into grains, corresponding to particle sizes below 45 μm [8]. The chemical composition of NS was determined in previous study [8].

2.2. Bacterial culture

M. barkeri was isolated from the sludge samples collected from the Shatin Sewage Treatment Works in Hong Kong, and characterized and identified by the MIDI Sherlock system and 16S rRNA gene sequence analysis. Gram stain was performed to identify Gram +ve and Gram −ve bacteria (Fig. S1 in the Supporting Information). The bacterial cells were cultured according to our previously published Ref. [12].

2.3. Photocatalytic inactivation

The detailed experimental procedures of photocatalytic inactivation for the two bacteria were described as following: The reaction mixture containing 1 g/L NS and 1.5×10^7 cfu/mL bacterial cells in saline (0.9% NaCl) solution was stirred with a magnetic stirrer (180 rpm) throughout the experiments. The unadjusted pH value of the mixture was 6.8. The fluorescent tubes (FTs, 15 W, VELOX®, Thailand) were chosen as the light source. The major wavelength is $\lambda \geqslant 400$ nm, with an intensity of 3.3 mW/cm^2 measured by a light meter (LI-COR®, Lincoln, Nebraska, USA) and UVA intensity of 0.03 mW/cm^2 measured by a UV meter (The light spectrum of the FT is included in Fig. S2 (in the Supporting Information). The temperature of the reactor was kept at around 25 °C. The bacterial cell density is monitored by plant count method described in our previous study [13] and the detection limit of spread plate was 1 CFU (colony forming unit)/mL. Three control experiments including light control (VL alone without photocatalyst), dark control (1 g/L photocatalyst alone without VL), and negative control (without VL or photocatalyst) were carried out. Each experiment was conducted in triplicates.

2.4. Analysis of zeta-potential

The zeta-potentials of NS and bacterial cells in NaCl solution (0.1 M) were measured with a ZetaPlus system (Brookhaven Instruments Co., New York) at 25 °C [14].

2.5. Analysis of reactive oxygen species

The scavenger experiments were carried out to study the role of reactive oxygen species (ROSs) by adding individual scavenger to reaction mixture before VL irradiation. 0.1 mmol/L Fe(II)-EDTA was used for scavenging H_2O_2 [15], 0.05 mmol/L $K_2Cr_2O_7$ for the quenching of e^- [16], 0.5 mmol/L isopropanol for the scavenging of bulk ·OH [17], 5 mmol/L KI for the scavenging of h^+ and ·OH bound to the surface of the photocatalyst (·OH_s) [18], and 2 mmol/L 4-hydroxy-2,2,6,6-tetramethyl-piperidinyloxy (TEMPOL) for removing $\cdot O_2^-$ [18].

Electron spin resonance (ESR) spectrometry (Brucker ESR A300 spectrometer, Rheinstetten, Germany) was used to record signals of superoxide radical ($\cdot O_2^-$) by the reaction with 5,5-dimethyl-1-pyrroline-N-oxide (DMPO). The settings for ESR spectrometer were center field of 3507 G, sweep width of 80 G, microwave frequency of 9.85 Hz, power of 6.34 mW, modulation frequency of 100 kHz, and modulation amplitude of 1 G. Terephthalic acid was used as a probe molecule to fluorescently detect the formation concentration of hydroxyl radical (·OH) in the NS system under VL irradiated [19,20]. Hydrogen peroxide was analyzed photometrically by the DPD/peroxidase method [21].

2.6. Transmission electron microscopy and measurement of potassium ion (K^+)

At different irradiation time intervals, the cell suspensions were collected, centrifuged and prepared for the TEM analysis according to the standard procedures [15]. To determine the leakage of K+ from the tested wastewater bacteria during the photocatalytic inactivation process, the concentration of K+ was measured by atomic absorption spectrophotometry (AAS) analysis on a Z-2300

Polarized Zeeman Atom Absorption Spectrophotometer (Hitachi High-Technologies, Tokyo, Japan). The sample was prepared according to that reported by Zhang et al. [15].

3. Results and discussion

3.1. Photocatalytic inactivation

Fig. 1 shows the photocatalytic inactivation of two wastewater bacteria by NS under VL irradiation at different pHs. The pH was adjusted at the beginning of the irradiation by addition of NaOH or HCl (1 mol/L) to the mixture. Inactivation to below detection limit of 1.5×10^7 cfu/mL of *M. barkeri* was observed within 10 h at pH 10 (Fig. 1A) and the inactivation efficiency declined with the reduction of the pH value from 10 to 5. However, in the dark and light controls, the bacterial population remained constant after 10 h treatment (Fig. S3 in the Supporting Information). The results indicate that the NS (dark control) or photolysis (light control) showed no toxic effect on *M. barkeri* at different pH values. Similar results were obtained for *E. coli*. as shown in Fig. 1B. Inactivation below the detection limit of 1.5×10^7 cfu/mL of *E. coli* was achieved within 3 h treatment at pH 10 and the inactivation efficiency also decreased with the decrease of the pH value from 10 to 5. For both wastewater bacteria, higher inactivation efficiency was achieved at higher pH values. Our previous study [12] showed that NS can be activated by blue and orange LED efficiently. Therefore, the inactivation efficiency should be greatly improved under sunlight irradiation as the light intensity in visible light is much higher compared to the light source used in the present study.

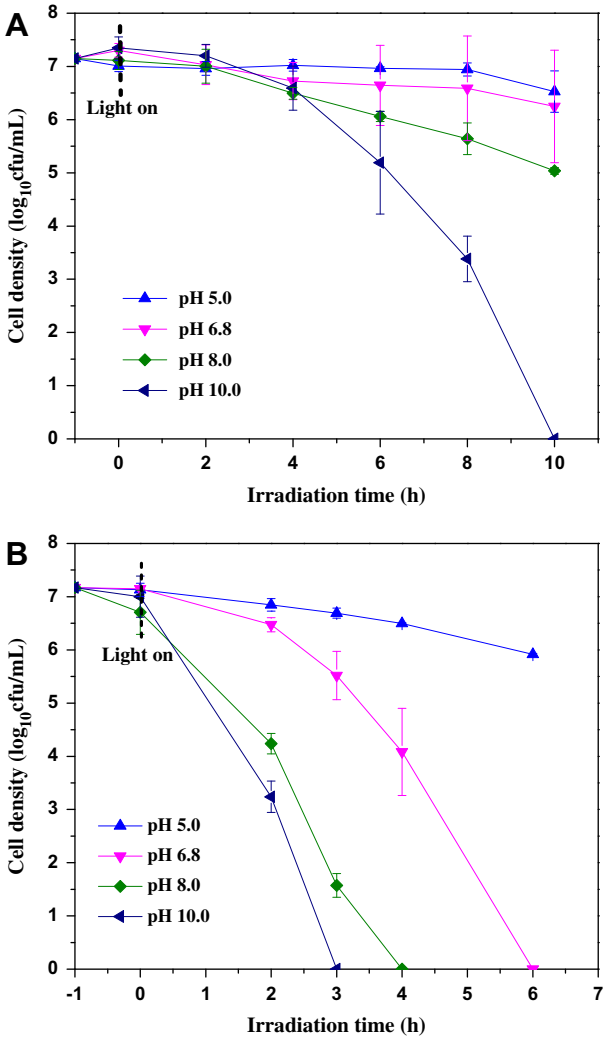

Fig. 1. Photocatalytic inactivation of (A) *M. barkeri* and (B) *E. coli* by NS under VL irradiation at different pH values. Experimental conditions: NS concentration = 1 g/L; Bacterial cell concentration = 1.5×10^7 cfu/mL. Each data point and error bar represents the mean and standard deviation, respectively, of independent triplicates.

Comparing the inactivation efficiency of both bacteria, the inactivation of *E. coli* is more effective than that of *M. barkeri*. The difference in inactivation between the two bacteria is due to the structural differences of the two bacteria as Gram +ve bacterium has a thicker cell wall than the Gram −ve bacterium [22], which will be more difficult to be attacked by photogenerated ROSs. This also agrees with the results from previous study which reported than Gram +ve bacteria are more resistant than Gram −ve bacteria in photocatalytic inactivation [9,10].

To determine whether electrostatic interaction influenced the inactivation efficiencies at different pH values, the zeta-potentials of NS and two wastewater bacteria were measured as a function of pH. As shown in Fig. 2, the point-of-zero-charge (PZC) of NS was approximately 3.5 pH units. In the range of pH 3.5–10, both the surfaces of NS and *E. coli* were negatively charged, whereas that of *M. barkeri* was nearly neutral (Fig. 2). In addition, the zeta-potentials of the samples were similar in the range of pH 5–10. These results suggest that this pH range, NS and the two wastewater bacteria were negatively charged, and the electrostatic attraction between the bacterial cell and NS can be excluded. Hence, the difference of the photocatalytic inactivation efficiencies are not due to the electrostatic interaction between bacterial cells and NS, but probably due to the different response content to ROSs generated during the photocatalytic process, which will be discussed further below.

3.2. Photocatalytic inactivation mechanism

3.2.1. Analysis of reactive oxygen species

The mechanism of ROSs formation by TiO_2 is well studied and reported in different review articles [23,24]. However, the ROS formation for photocatalytic inactivation by NS may be different from that reported in TiO_2. To investigate which ROSs are involved in the inactivation of *M. barkeri* and whether there is a difference response between Gram +ve and Gram −ve bacteria in the photocatalytic inactivation, the scavenger experiments were carried out to remove the respective ROS so as to study the importance of individual ROS in the system. In the dark and light controls, after the

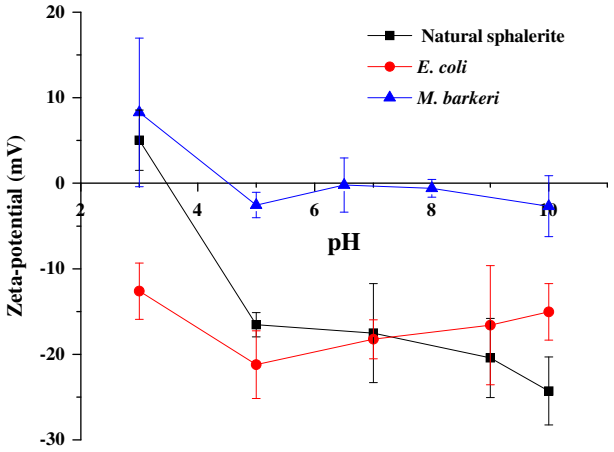

Fig. 2. Zeta potentials for suspensions of NS, *M. barkeri* and *E. coli* in the presence of NaCl (0.1 M).

addition of each scavenger, the bacterial population of *M. barkeri* remained constant within 10 h (data not shown), suggesting that the concentration of the scavengers used in the present study is not toxic to the selected bacteria. Without any scavengers, the 5 log reduction of 1.5×10^5 cfu/mL *M. barkeri* was achieved within 10 h at neutral pH. The addition of KI, isopropanol, TEMPOL and Cr(VI), did not significantly inhibit bacterial inactivation efficiency (Fig. 3), indicating that h^+, bulk ·OH, surface ·OH, $\cdot O_2^-$ and e^- are not directly involved in the photocatalytic inactivation of *M. barkeri*. Interestingly, in the presence of Fe(II) to remove H_2O_2, the photocatalytic inactivation efficiency was greatly inhibited in which only 1 log reduction is observed (Fig. 3). This suggests that H_2O_2 is greatly involved in photocatalytic inactivation of *M. barkeri*.

To further clarify the role of e^-, four scavengers (isopropanol, KI, Fe(II), and TEMPOL) were added together to remove h^+, ·OH, H_2O_2, and $\cdot O_2^-$ simultaneously. In this case only e^- remained in the inactivation system. The results show that in the presence of e^-, the bacterial cell density did not change in the first 8 h, and only slight decrease was observed (less than 0.5 log) after 10 h treatment (Fig. 3). The result indicated that it is not the photogenerated e^- but H_2O_2 play the major role in the photocatalytic inactivation of *M. barkeri*. The conclusion is quite different from that of *E. coli* which both e^- and H_2O_2 are important in the photocatalytic inactivation of *E. coli* [13]. Gao et al. [25] have proposed a mechanism of bacterial inactivation by photogenerated e^- which involved the activation of fatty acid by coenzyme-A. Therefore, it is possible that the difference of inactivation mechanism between the two wastewater bacteria is related to the difference in cell membrane composition.

To further confirm the role of ROSs in photocatalytic inactivation of *M. barkeri*, ESR technique was applied to examine $\cdot O_2^-$, and fluorescent and photometrical measurements were used to detect ·OH and H_2O_2, but no $\cdot O_2^-$ signal was detected under VL irradiation (Fig. S4). It is probably that $\cdot O_2^-$ is not produced by NS-VLD system or the amount of $\cdot O_2^-$ is relatively too low to be detected. This result agrees well with the scavenger study and confirms that $\cdot O_2^-$ is not an important ROS for the photocatalytic inactivation of *M. barkeri*. Although it is commonly accepted that the H_2O_2 produced from the conduction band, the results suggest that the H_2O_2 generation by NS should be the direct reaction between e^- and O_2 ($O_2 + 2\ e^- + 2\ H^+ \rightarrow H_2O_2 + 0.682$ eV) as very limited $\cdot O_2^-$ is detected [26].

Furthermore, although the fluorescent measurement of ·OH (Fig. S5) indicates that ·OH could be produced with a little amount in the suspension of NS under VL excitation, the addition of isopropanol demonstrates that ·OH is not mainly involved in photocatalytic inactivation of *M. barkeri*. The finding is quite different to the inactivation mechanism of Gram −ve bacteria as previous study reported that ·OH play an important role inactivation of *E. coli* [15]. Furthermore, the amount of H_2O_2 produced by NS was determined at different pH values. Fig. 4 shows the absorption spectra of the DPD/POD after reaction with H_2O_2 after 2 h irradiation at pH values of 6.8 and 10. Obviously, the absorbance (at 551 nm) at pH 10 was higher than that of pH 6.8, which suggests that more H_2O_2 is produced at more alkaline pH. It is probably that the higher inactivation efficiency obtained at alkaline pH is due to the higher amounts of H_2O_2 produced by NS. This confirms the importance of H_2O_2 in photocatalytic inactivation of *M. barkeri*. Although a low concentration of the H_2O_2 is detected (several μmol/L) in the NS system, the actual amount of H_2O_2 produced in the system is much more than actually measured value as H_2O_2 is continuously consumed by the bacterial cell [27]. Kukichi et al. [28] also discovered the effective inactivation of bacterial cells with low concentration of H_2O_2. They suggested that there may be a cooperative effect between the H_2O_2 and other ROSs which may also happen in this system. Therefore, H_2O_2 is important in the inactivation process even with a low concentration detected.

3.2.2. Photocatalytic destruction of *M. barkeri*

To further confirm the destruction process, the morphology of *M. barkeri* was studied by TEM before and after photocatalytic inactivation. Fig. 5 shows the inactivation of *M. barkeri* at different stages of photocatalytic treatment. Before inactivation, a small rod shaped of *M. barkeri* exhibited an intact cell structure including the obvious cell wall and evenly rendered interior of the cell (Fig. 5A). After 10 h irradiation treatment (Fig. 5B), the central portion of the cell was still intact but part of the cell wall structure appeared damage. Upon irradiation for 30 h (Fig. 5C), more cell wall structure disappeared and a small part of the interior components of the bacteria became translucent. After 50 h of irradiation treatment (Fig. 5D), the bacterial cell was almost completely ruptured. The TEM results suggest that the inactivation process of *M. barkeri* starts from the cell wall.

Compared with *M. barkeri*, the cell size of *E. coli* is much bigger and present in long-rod shaped (Fig. 6). Only after 6 h photocatalytic inactivation, *E. coli* showed obvious cell membrane damage

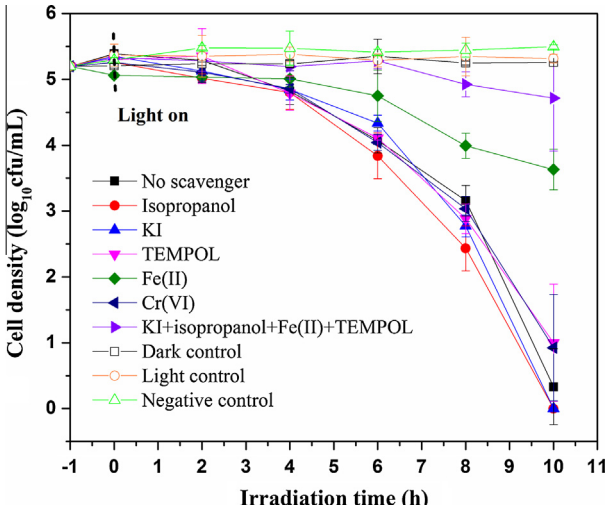

Fig. 3. Photocatalytic inactivation of *M. barkeri* by NS under VL irradiation at neutral pH with different scavengers (5 mmol/L KI, 0.5 mmol/L Isopropanol, 0.05 mmol/L Cr(VI), 0.1 mmol/L Fe(II)-EDTA, 2 mmol/L TEMPOL).

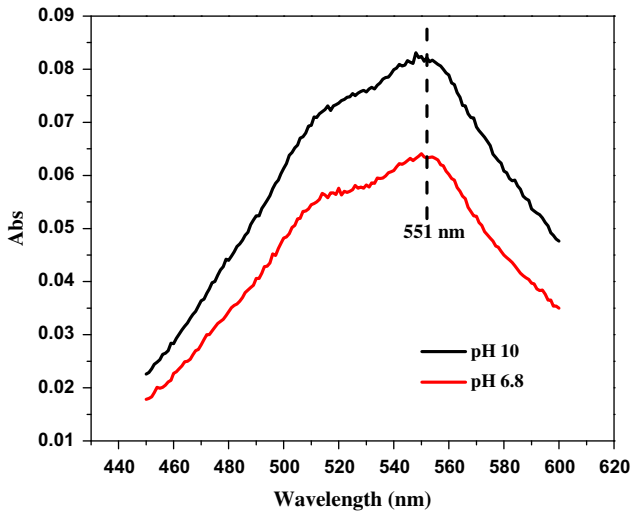

Fig. 4. Absorption spectra of the DPD/POD after reaction with H_2O_2 produced by the VL irradiation for 2 h at different pH values.

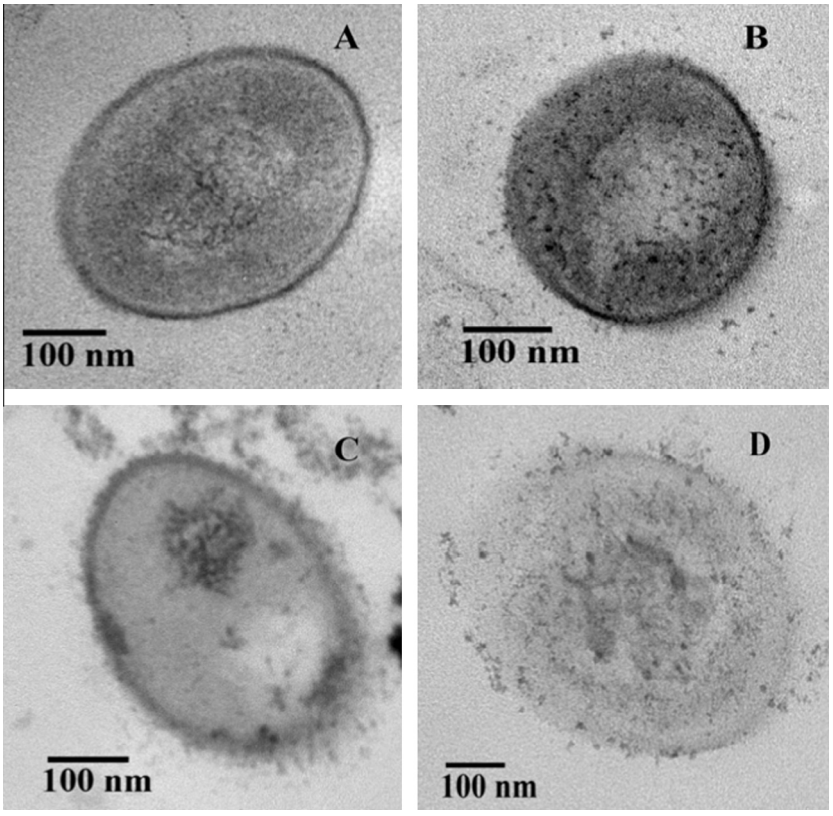

Fig. 5. TEM images of *M. barkeri* photocatalytically treated with NS under VL irradiation. (A) 0 h, (B) 10 h, (C) 30 h, and (D) 50 h.

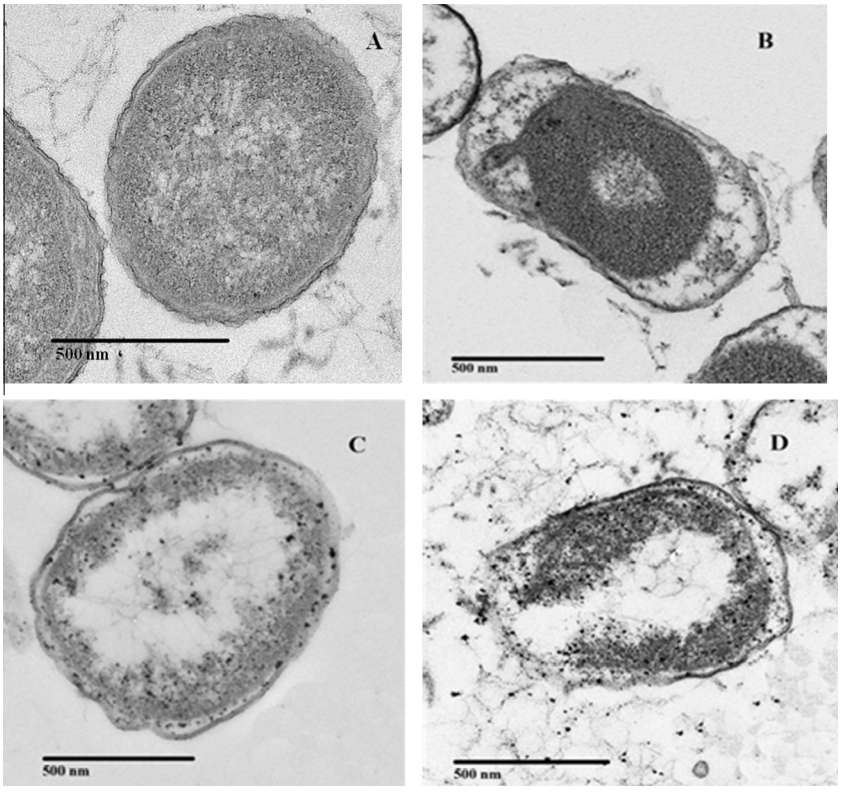

Fig. 6. TEM images of *E. coli* photocatalytically treated with NS under VL irradiation. (A) 0 h, (B) 6 h, (C) 12 h, and (D) 30 h.

(Fig. 6B). Most of the interior components have been degraded and became translucent after 12 h photocatalytic treatment (Fig. 6C) and further destruction was observed at 30 h irradiation (Fig. 6D). The above illustrations of bacterial morphological changes indicate that, the photocatalytic inactivation process is similar for the two bacteria; both of them are damaged from the cell wall. But the destructive process of *E. coli* is much faster than *M. barkeri*. Since the inactivation of both bacteria started at the cell envelope (i.e. cell wall and cell membrane), this also suggests that the difference in major ROS involved between the two bacteria is related to their cell envelope structures.

To further confirm the destruction of cell membrane, K^+ leakage measurements were conducted for two bacteria (Fig. S6 in the Supporting Information) as K^+ actually exists in bacterial cell and is involved in the regulation of polysome content and protein synthesis [2,29]. Any damage in the cell membrane structure during the photocatalytic inactivation will cause the leakage and accumulation in the solution of K^+ because of the changes of permeability of cell membrane and the loss of cell viability.

4. Conclusions

Natural sphalerite could photocatalytically inactivate both Gram-positive (+ve) bacterium *M. barkeri* and Gram-negative (−ve) bacterium *E. coli* under visible light. H_2O_2 was found to play a significant role in *M. barkeri* inactivation, while both H_2O_2 and e^- were responsible for *E. coli* inactivation. The structural difference between Gram +ve and Gram −ve bacteria lead to the differences in inactivation efficiencies and mechanisms, that Gram −ve bacterium with a thinner cell wall was more susceptible to be damaged by photogenerated radicals. Results of this study will help optimize engineering parameters in future application of natural sphalerite in large-scale wastewater disinfection.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.cej.2013.08.106.

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