Photoelectrocatalytic Inactivation of *E. coli* by ZnSe/CdS Co-sensitized TiO₂ Nanotube Array

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A step-wise structural ZnSe/CdS co-sensitized TiO₂ nanotube (NT) electrode was prepared by pulse-electrodeposition and successive ionic layer adsorption and reaction (SILAR), with an aim towards enhancing the absorption efficiency of TiO₂ NTs in visible light region and enhancing the photo-electrical transform efficiency. The as-prepared CdS/ZnSe/TiO₂ NTs were applied to the photoelectrocatalytic inactivation of *E. coli*. A complete inactivation of 3.0×10^8 cfu mL⁻¹ *E. coli* was observed after 60 min of photoelectrocatalysis process under the illumination of visible light.

Keywords TiO₂ nanotube array, ZnSe, CdS, photoelectrocatalytic inactivation, *E. coli*

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Introduction

Strains of *E. coli* are gram-negative bacteria. The majority of them are beneficial to the host, but a few of them can cause distinct diarrhea syndromes. In 1982, *E. coli* O157:H7 was first recognized as a human pathogen because it can cause urinary tract infections, hemolytic uremic syndrome and thrombotic thrombocytopenic purpura in humans.¹ Furthermore, *E. coli* O157:H7 attracted attention with regard to food safety due to its pathogenicity, the low infectious dose of *E. coli* O157:H7 (less than 50 organisms), the severity of illnesses caused by *E. coli* O157:H7 and the nature of susceptible populations most affected by *E. coli* O157:H7 infection.² Thus, it is very necessary to develop an effective material for inactivation of *E. coli*.

Titanium dioxide (TiO₂) nanomaterials have attracted great attention because of its high stability, non-toxicity, high oxidative power and low cost.³⁻⁶ TiO₂-based photocatalysts have been used to inactivate bacteria, viruses, and cancer cells.⁷⁻¹² However, the wide band gap (3.2 eV) of TiO₂ means it can only absorb spectrum in the near-UV region, which limits its, practical applications for photocatalytic inactivation of bacteria. There is thus a need to enhance the absorption capability of bactericidal materials in the visible region. To achieve this, much effort has been directed at modifying of TiO₂ with nonmetal anions,¹³ metallization¹⁴ and narrow-band gap semiconductors.¹⁵ It has been well established in recent research that combining wide-band gap TiO₂ with small-band gap nanocrystals, such as CdS,¹⁶ CdSe,¹⁷ ZnSe,¹⁸ and Cu₂O¹⁹ can render TiO₂ sensitive to visible light.

In this paper, a TiO₂ (3.2 eV) nanotube was modified with ZnSe (2.67 eV) and CdS (2.25 eV) by two steps; pulse-electrodeposition, and successive ionic layer adsorption and

reaction (SILAR). The as-prepared compound absorbs solar-photons efficiently and inhibits the recombination between photo-generated electrons and holes. The CdS/ZnSe/TiO₂ NTs electrode showed significant enhanced photoelectrocatalytic (PEC) activity under visible light irradiation and proved to be effective for inactivation of *E. coli*.

Results and Discussion

Characterization of the as-prepared materials

Figure 1A shows the typical SEM images of the TiO₂ NTs

Fig. 1 (A) The cross-sectional SEM images of CdS/ZnSe/TiO₂ NTs with top view inset. (B) SEM images of top view of the ZnSe(20)/TiO₂ NTs photoelectrode; (C) SEM images of CdS(5)/ZnSe(20)/TiO₂ NTs; (D) EDX spectrum of ZnSe(20)/CdS(5)/TiO₂ NTs.

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Fig. 2 (A) Photocurrent responses of (a) bare TiO₂ NTs; (b) ZnSe(20)/TiO₂ NTs; (c) CdS(5)/TiO₂ NTs; (d) CdS(4)/ZnSe(20)/TiO₂ NTs; (e) CdS(6)/ZnSe(20)/TiO₂ NTs; (f) CdS(5)/ZnSe(20)/TiO₂ NTs. (B) UV-Vis diffuse reflectance spectrum of (a) pure TiO₂ NTs; (b) ZnSe(20)/TiO₂ NTs; (c) CdS(5)/TiO₂ NTs and (d) CdS(5)/ZnSe(20)/TiO₂ NTs. (C) I-V characteristics for pure (a) TiO₂ NTs; (b) ZnSe(20)/TiO₂ NTs; (c) CdS(5)/TiO₂ NTs and (d) CdS(5)/ZnSe(20)/TiO₂ NTs electrodes.

with an average length of 400 nm, an inner pore diameter ranging from 70 to 110 nm and a wall thickness of about 15 nm. As can be seen from Fig. 1B, ZnSe nanoparticles (NPs) with an average size of about 26 nm are dispersed on the top surface of the TiO₂ NTA, which would make the photoelectrode sensitive to visible light. Following the initial deposition of ZnSe NPs, CdS NPs were successfully deposited on the ZnSe/TiO₂ NTA by SILAR, Fig. 1C. The ZnSe/TiO₂ NTs are fully covered with CdS NPs. And it is clearly seen that some CdS NPs have been deposited into the pore of the ZnSe/TiO₂ NTs. The energy dispersive X-ray spectroscopy (EDX) analysis (Fig. 1D) exhibits the characteristic peaks of O, Zn, Se, Cd, S and Ti in CdS(5)/ZnSe(20)/TiO₂ NTs with a molar ratio of Zn to Se of 1.67:1 and a molar ratio of Cd to S of 1:1.

Photoelectrochemical behavior of CdS/ZnSe/TiO₂ NTs

Figure 2A shows the photoelectrochemical behaviors of CdS/ZnSe/TiO₂ NTs, ZnSe/TiO₂ NTA, CdS/TiO₂ NTs and pure TiO₂ NTs using a three-electrode PEC cell in sulfide-sulfite (S²⁻/S₃²⁻) electrolyte under AM 1.5G illumination. The maximum photocurrent density of CdS(5)/ZnSe(20)/TiO₂ is 37.14, 16.59 and 1.46 times that of the bare TiO₂ NTs, ZnSe(20)/TiO₂ NTs and CdS(5)/TiO₂ NTs photoelectrodes, respectively. The significant enhancement in photocurrent is mainly attributed to the synergistic effect of ZnSe and CdS within CdS/ZnSe/TiO₂ NTs. On the one hand, the decoration of ZnSe and CdS onto TiO₂ nanotube arrays renders the TiO₂ NTA sensitive to visible light and improves utilization of the visible light, which means more photons can be captured for producing the electron-hole pairs.²⁰ This conclusion is supported by UV-vis absorption studies in Fig. 2B. Obviously, the anchoring of ZnSe and CdS to TiO₂ NTs results in an enhancement in the absorption spectrum in the visible region of the CdS/ZnSe/TiO₂ NTs and a red shift. On the other hand, because of the proper matching of the energy levels, ZnSe and CdS are applied to construct an appropriate step-wise structure in the CdS/ZnSe/TiO₂ NTs photoelectrode.³² Such a structure is advantageous not only for transport of the photogenerated electron but also for the separation of the excited electron-hole pairs. Based on the above reasons, a significant enhancement of photocurrent is displayed in the CdS/ZnSe/TiO₂ NTs electrode. The CdS/ZnSe/TiO₂ NTs therefore show the highest photocurrent density also in the photocurrent-applied potential relationships (I-V), Fig. 2C.

Antibacterial test

Figure 3A compares the PEC antibacterial activity of *E. coli* for different samples. Under similar conditions, the CdS/ZnSe/TiO₂ NTs electrode exhibited the highest PEC activity for inactivation of *E. coli*. An almost complete inactivation of *E. coli* was observed after 60 min of irradiation, which is higher than those of the ZnSe/TiO₂ NTs electrode (2.48 logs removal of *E. coli*) and pure TiO₂ NTA electrode (1.6 logs removal of *E. coli*). The high PEC activity is attributed to the high absorption coefficient in the visible region of ZnSe and CdS.

Figure 3B shows the inactivation of *E. coli* on the CdS/ZnSe/TiO₂ NTs electrode during different killing processes, including direct photolysis, electrochemical oxidation, photocatalysis and photoelectrocatalysis. The direct photolysis (without any photocatalyst) with visible light (300 – 800 nm) resulted in little decrease in *E. coli*. The photocatalysis (without a bias potential) of the CdS/ZnSe/TiO₂ NTs electrode inactivated 2.4 logs of *E. coli* during the same time. In the electrochemical oxidation (with a bias potential of 0.6 V but no illumination), the concentration of *E. coli* decreased by 3.2 logs. The photoelectrocatalysis (PEC) process killed all *E. coli* within 60 min of irradiation with a bias potential of 0.6 V. The above results indicated that the CdS/ZnSe/TiO₂ NTs electrode was highly effective at killing *E. coli* under visible light irradiation. The applied bias potential could improve the electrosorption of bacteria on the electrode surface which is beneficial to sterilization.²¹⁻²³ The pictures of *E. coli* colonies on an agar plate before and after PEC inactivation treatment for different times are shown in Fig. 3D; the viable colonies on the plates decreased significantly with increasing the irradiation time, and a complete inactivation of *E. coli* could be observed after 60 min of treatment.

According to the published works^{16,31} and the above experimental results, the mechanisms of bactericidal reaction over the CdS/ZnSe/TiO₂ system was hypothesized as bellow, and illustrated in Scheme 1.

Fig. 3 (A) Inactivation of *E. coli* by PEC technology with pure TiO₂ NT, ZnSe/TiO₂ NTs and CdS/ZnSe/TiO₂ NTs electrodes under visible light irradiation. (B) Inactivation of *E. coli* by direct photolysis, electrochemical oxidation, photocatalysis, and photoelectrocatalysis, respectively, with the CdS/ZnSe/TiO₂ NTs electrode under visible light irradiation. (C) Changes of FT-IR spectra of *E. coli* during the photoelectrocatalysis inactivation process. (D) Images of *E. coli* colonies on an agar plate before and after PEC inactivation treatment with CdS/ZnSe/TiO₂ NTA electrode for 10, 20, 30, 40, 50 and 60 min.

Scheme 1 Illustrative diagrams of the electron and hole transfer in CdS/ZnSe/TiO₂ NTs and their effects on bactericidal reaction.

When TiO₂, ZnSe and CdS are connected together, the Fermi level of ZnSe shifts down with respect to the Fermi level

location in TiO₂, and the conduction band edge of CdS is elevated because of the Fermi level alignment.²⁴ As a result, the final conduction band edges within the CdS/ZnSe/TiO₂ NTs are constructed to be a step-wise structure as shown in Scheme 1, which facilitates the transfer of photogenerated electrons and avoids the recombination of electron-hole pairs. Due to the narrow band gap, visible light can be absorbed by either phase. The photogenerated electrons transfer from the conduction band of CdS to that of ZnSe with the help of a driving force. Finally, electrons arrive at the conduction band of TiO₂ and react with the adsorbed oxygen to yield O₂^{•-} which can combine with H⁺ to produce HO₂[•].^{25,26} Next, HO₂[•] reacts with the trapped electrons to generate OH[•] radicals.²⁷ Meanwhile, the photogenerated holes transfer from the valence band of TiO₂ to that of ZnSe, and finally accumulate in CdS to react with surface-bound H₂O producing OH[•] radical species.

To confirm the existence of OH[•] radicals, the fluorescent probe (terephthalic acid) was chosen to react with OH[•] radicals to form highly fluorescent 2-hydroxy terephthalic acid. The process was performed in an electrolyte containing 5 × 10⁻⁴ M terephthalic acid and 2 × 10⁻³ M NaOH. After 30 min of illumination, the photo luminescence (PL) intensity observed on TiO₂-ZnSe-CdS NTs is higher than that on TiO₂-ZnSe NTs and bare TiO₂ NTA (Fig. S1A), indicating more OH[•] radicals generated on TiO₂-ZnSe-CdS NTA than the other two electrodes at the same time. Figure S1B shows that the PL intensity of the TiO₂-ZnSe-CdS NTs increases gradually with increasing irradiation time. The above results indicate that OH[•] radicals are formed during the photoelectrochemical process and are the main killer towards *E. coli* in bacterial inactivation.

E. coli is protected by cell walls and cell membrane to

maintain an intact cell morphology and a stable internal environment. The cell wall contains an outer membrane and peptidoglycan (PGN) layer. The main components of the outer membrane are lipopolysaccharide (LPS), proteins and a phospholipid layer. The PGN constructed by glycan chains, short peptides and peptide bridge is bound to the outer membrane lipoprotein *via* a peptide bond. The glycan chains are made out of a polymer comprised of two amino sugars, *N*-acetylglucosamine (GNAc) and *N*-acetylmuramic acid (MurNAc). The cell membrane contains a phospholipid layer and protein. Also, 70 – 80% of the total pool of phospholipids of *E. coli* is phosphatidylethanolamine (PE). The remaining phospholipids are phosphatidylglycerol (15 – 20%) and cardiolipin (<5%).²⁸ Once inactivation of *E. coli* is performed by PEC process (with bias potential of 0.6 V) in the presence of CdS/ZnSe/TiO₂ NTs under solar light illuminated, the reactive species (OH[•], O₂^{•-} and holes) attack the cell membrane and wall of *E. coli* and disrupt membrane integrity, or electrolyze the molecules in the cell surface.²⁹ Figure 3C shows the FT-IR spectra of *E. coli* as a function of time during the PEC process. The characteristic peak of amide A appears at 3299 cm⁻¹ and amide B at 3299 and 3063 cm⁻¹.³⁰ The peaks at 2964, 2924, and 2861 cm⁻¹ were attributed to the vibration bands of $\nu_a(\text{CH}_3)$, $\nu_a(\text{CH}_2)$ and $\nu_s(\text{CH}_2)$, respectively. The peak of 1658 cm⁻¹ well matched the assignment for $\nu(\text{C}=\text{O})$ stretching vibrations. The amide II bands at 1542 cm⁻¹ contributed to N-H bending with contributions from the C-N stretching vibrations of the peptide group.³¹ The nucleic acid bands were observed near 1240 cm⁻¹, overlapping with the asymmetric stretching mode $\nu_{as}(\text{PO}_4^{2-})$ of the phospholipid phospho-diester.³² Moreover, the peak of 1088 cm⁻¹ was assigned to the vibrations of the sugar rings of lipopolysaccharides. With the irradiation time increasing, all of these characteristic peaks significantly decayed in intensity or even disappeared, which indicated that the reactive species sterilize bacteria and lead to leakages of the carboxylic acid during the photoelectrocatalysis process.

Conclusions

A step-wise structural ZnSe/CdS QD-sensitized TiO₂ NTs photoelectrode was prepared by pulse-electrodeposition and successive ionic layer adsorption and reaction (SILAR). The photon absorption efficient and photocurrent intensity of this as-prepared electrode were greatly enhanced due to the modification of the narrow band gap compounds of ZnSe and CdS, which constructed a step-wise conduction band facilitating the transportation of photogenerated electrons and suppressed the recombination of the electron-hole pairs. High photoelectrochemical activity in the inactivation of *E. coli* was therefore achieved. A complete inactivation of 3.0×10^8 cfu mL⁻¹ *E. coli* was observed after 60 min of photoelectrocatalysis process. And it was found that the photocatalytically generated OH[•] radical plays an important role in bactericidal performance.

Supporting Information

Experiment; PL spectra of pure TiO₂ NTA, TiO₂-ZnSe NTs and TiO₂-ZnSe-CdS NTs in 5×10^{-4} M terephthalic acid and 2×10^{-3} M NaOH under visible light irradiation at a fixed 30 min (Fig. S1A); PL spectra change with irradiation time on TiO₂-ZnSe-CdS NTs in 5×10^{-4} M terephthalic acid and 2×10^{-3} M NaOH (Fig. S1B). This material is available free of charge on the Web at <http://www.jsac.or.jp/analsci/>.

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