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Visible-light photocatalytic inactivation of *Escherichia coli* by $K_4Nb_6O_{17}$ and Ag/Cu modified $K_4Nb_6O_{17}$

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ABSTRACT

Ag/Cu modified $K_4Nb_6O_{17}$ thin film, a novel type of photocatalyst for photocatalytic inactivation of *Escherichia coli* under visible light irradiation was developed. The effects of loading method of Cu species on the characteristics of Ag–Cu nanocomposites and photocatalytic antibacterial activity were studied. Samples were characterized by powder X-ray diffraction (XRD), transmission electron microscopy (TEM), X-ray photoelectron spectroscopy (XPS) and ultraviolet–visible spectroscopy (UV–Vis). Irrespective of the preparation method, loading Ag, Cu and Ag–Cu composite as cocatalyst led to an increase in antibacterial activity as compared to bare $K_4Nb_6O_{17}$. In comparison with conventional impregnation method, well dispersed Ag–Cu nanocomposites were obtained on the $K_4Nb_6O_{17}$ surface by a sodium borohydride reduction method in the presence PVP. In this case, markedly improvement of photocatalytic activity was observed. The significant enhancement was ascribed to the high efficiency of electron hole pair separation related to the Ag–Cu nanocomposite on $K_4Nb_6O_{17}$ surface and the synergistic effects of coexisting Ag and Cu ions on antibacterial activity.

1. Introduction

Our world is always under threat from biological contamination. Recent outbreaks of virulent *Escherichia coli* contamination in Germany have demonstrated the urgent need worldwide to control the harmful pathogen. Developing a photocatalytic system for antibacterial control using light is a topic of great interest, and is of fundamental as well as practical importance. The photocatalytic deactivation of microorganisms is a cheap, clean and safe alternative as compared to chemical disinfections such as chlorine, iodine and ozone treatment. When a semiconductor photocatalyst is illuminated with photons with energies greater than its band gap, electron–hole pairs are generated due to photo-excitation. Highly oxidative hydroxyl radicals are generated by the oxidation of water at the valence band of semiconductor photocatalyst whose valence band level is more positive than the oxidation potential of H_2O. Further, oxygen is reduced to reactive oxygen species such as superoxide anion and hydrogen peroxide by the photo excited electrons of the photocatalyst whose conduction band edge should be more negative than the reduction potential of O_2 [1]. Although the mechanism of antibacterial action has not been fully elucidated, it is believed that the reactive oxygen species produced by the photocatalytic reactions cause various damages to microorganisms [2,3].

As it is well known, titanium oxide is the most widely used photocatalyst for wastewater treatment and destruction of volatile organic compounds. TiO_2 photocatalyst has also shown to be effective for bacterial inactivation under UV light irradiation. However, the incoming solar energy contains only about 4% UV energy. On the other hand, exposure to UV light would cause damage to human skin [4]. Hence, applications of photocatalytic disinfection driven by UV light are significantly limited. Many studies have been made to develop highly efficient photocatalysts for bacterial inactivation driven by visible light. Yu et al. [5] reported that sulfur-doped titanium dioxide showed visible-light induced photokilling capability on *Micrococcus lylae* (gram-positive) cells where the survival ratio of *M. lylae* decreased to 3.3% after 60 min irradiation. Recently, Wu et al. [6] reported that the combination of Ag_2O and TiON photocatalysts showed high efficiency on the photokilling of *E. coli* (gram-negative) in a suspension photoreactor.

Furthermore, the activity of photocatalyst could be improved by loading cocatalyst, such as Pt, Cu, Ag, and ZnO [7–9]. Tseng et al. [7] reported that significant enhancement effects of loading Cu on TiO_2 for the photoreduction of CO_2. Page et al. [10] reported that Ag modified TiO_2 was significantly more photocatalytically and antimicrobially active than TiO_2. In addition, silver ion is known as a good antibacterial agent [15,16]. Silver sulfadiazine cream is one of the most widely used for the managing burns [11]. Ag ions can

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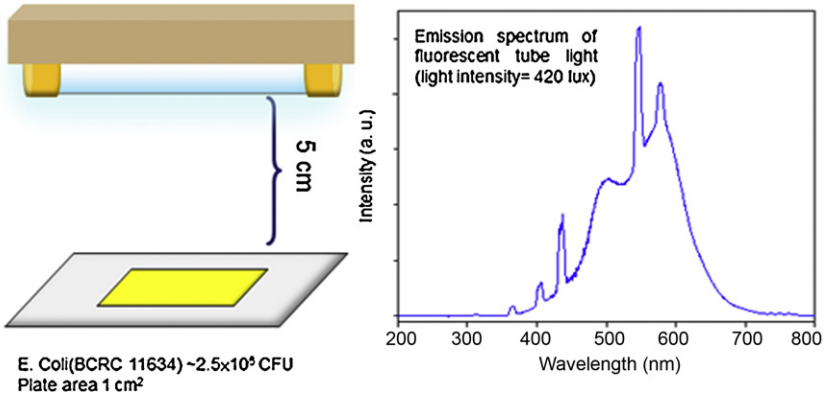

Fig. 1. Schematic diagram of antibacterial test.

interact with the thiol groups in protein and subsequently inactivated of key enzymes. Also, Ag ions could kill bacteria by denaturing their DNA molecular [12]. Several comprehensive reviews have reported on the aspects of the disinfection mechanisms [13] and photocatalytic antibacterial mechanisms [14].

On the other hand, much effort has been devoted to studying the photocatalytic properties of transition metal oxides with a d^0 electron configuration such as $InNi_{0.1}TaO_4$ [15], $K_4Nb_6O_{17}$ [16,17], and $BiVO_4$ [18] for hydrogen production from water splitting under visible light irradiation. Nevertheless, very few studies have investigated the photocatalytic disinfection of non-TiO_2 photocatalysts. Previously, our group had reported that highly crystalline single phase $K_4Nb_6O_{17}$ can be prepared by a two-step solid-state reaction process and showed photocatalytic activity for water splitting under visible light [16]. $K_4Nb_6O_{17}$ consists of four layers of NbO_6 orthorhombic units where the anisotropic niobate sheets stacked along the b axis form two types of interlayer regions, which are denoted by Interlayer I and Interlayer II [19]. $K_4Nb_6O_{17}$ may be a good candidate for photocatalytic disinfection under visible light due to its anisotropic layered structure for efficient charge separation.

In the present study, we present a series of novel $K_4Nb_6O_{17}$ thin film photocatalysts with Ag, Cu, Ag–Cu cocatalysts for photocatalytic disinfection under visible light irradiation. Individual and combined effects of Cu and Ag cocatalysts on $K_4Nb_6O_{17}$ were investigated. Special attention has been focused on the effects on the relationship between loading method of Ag–Cu nanoparticles and photocatalytic disinfection activity. The catalysts were studied using powder X-ray diffraction (XRD), transmission electron microscopy (TEM), ultraviolet–visible spectroscopy (UV–Vis), scanning electron microscopy–energy dispersive X-ray spectrometer (SEM–EDX) and X-ray photoelectron spectroscopy (XPS). The antibacterial activity of photocatalyst film was evaluated under the Japanese standard JIS Z 2801: 2006 [20].

2. Experimental

The preparation of $K_4Nb_6O_{17}$ catalysts were synthesized by a two-step solid-state reaction (SSR) using K_2CO_3 and Nb_2O_5 with 99.99% purity (molar ratio 2.1:3). The mixed precursor was calcined in air at 1073 K for 5 h then at 1273 K for 5 h with an intermediate grinding process between the two calcinations.

The Ag–$K_4Nb_6O_{17}$, Cu–$K_4Nb_6O_{17}$ catalyst was prepared by loading 1 wt% Ag and 1 wt% Cu on $K_4Nb_6O_{17}$ powders using aqueous $AgNO_3$ and $Cu(NO_3)_2$ solution, respectively.

Ag–Cu(1) $K_4Nb_6O_{17}$ catalyst was prepared by loading 0.5% Ag and 0.5 wt% Cu on $K_4Nb_6O_{17}$. Distilled water containing an appropriate amount of $AgNO_3$ and $Cu(NO_3)_2$ was premixed before the impregnation process. Following the impregnation process, the film of Ag–$K_4Nb_6O_{17}$, Cu–$K_4Nb_6O_{17}$ and Ag–Cu(1) $K_4Nb_6O_{17}$ was prepared with the same protocol as following: 0.15 g photocatalyst was dispersed in 1 ml of distilled water to make a slurry and subsequently coated on glass substrate via a doctor-blade method with an active area about 1 cm^2. The film was dried for 30 min on a hot plate at 80 °C and subsequently calcined at 400 °C for 70 min.

Ag–Cu(2) $K_4Nb_6O_{17}$ and Ag–Cu(3) $K_4Nb_6O_{17}$ catalyst were prepared by loading 0.5 wt% Cu on $K_4Nb_6O_{17}$ by dissolving appropriate amount of $CuSO_4{\cdot}5H_2O$ in 25 ml of distilled water where 0.7 g PVP (polyvinyl pyrrolidone, M_{av} = 55,000) had previously been added. The mixture was stirred under nitrogen for 20 min. Then, 25 ml 0.01 M aqueous solution of sodium borohydride was added to the solution and further reacted under nitrogen for 20 min. After centrifugation and washing, the resulting product was dried at 60 °C overnight. Subsequently, 0.5 wt% Ag was loaded on the product by impregnation process. The film was prepared by the same protocol as described above. The Ag–Cu(2) $K_4Nb_6O_{17}$ film was referred as the sample before calcination. After calcined at 400 °C for 70 min, the sample was denoted as Ag–Cu(3) $K_4Nb_6O_{17}$. The Cu and Ag loading were measured by inductively coupled plasma mass spectrometry (ICP-MS, see Supplementary data).

The characterization methods included powder X-ray diffraction (XRD, Cu Kα radiation, λ = 1.54178 Å), TEM (JEOL JEM-2000FX Π microscope), SEM/EDS (Hitachi S-3500H), and UV–Vis (Varian Lary 5E diode array spectrometer). The XPS spectra were recorded with a VG Scientific ESCALAB 250. The binding energy of XPS were corrected by contaminant carbon (C_{1s} = 284.6 eV) in order to facilitate the comparisons of the values among the catalysts and the standard compounds.

The antibacterial activity of photocatalyst film was evaluated under the Japanese standard JIS Z 2801: 2006 [20]. *E. coli* (BCRC 11634) was incubated in Luria–Bertani (LB) broth at 37 °C for 12–16 h at relative humidity ≥95%. The *E. coli* was grown to $1–3 \times 10^9$ colony-forming units (CFU/ml) which was determined by the spectroscopy enumeration method, and then the cell suspension was diluted to about 1.0×10^7 to 5.0×10^7 CFU/ml in LB broth. 50 μl of cell suspension (about 2.5×10^6 CFU) was then plated on the sample specimens. The samples were illuminated by two 10 W fluorescent tube light (China Electric MFG. Corporation, FL10D, 420 lux) for 45 min as shown in Fig. 1. Specimens on glass substrate without photocatalyst were carried out as control experiments. The sample specimens treated by the same protocol with no light irradiation was used as dark control experiments. All the photocatalytic experiments were repeated three times. The antibacterial test was effective while: (i) $(\log(N_{max}) - \log(N_{min}))/\log(N_{avg}) \leq 0.2$, where N_{max}, N_{min}, and N_{avg} was the maximum, minimum and average number of viable cells, respectively. (ii) The average of the