Photocatalytic bacterial inactivation by polyoxometalates

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Abstract

The photocatalytic inactivation (PCI) of *Escherichia coli* (Gram-negative) and *Bacillus subtilis* (Gram-positive) was performed using polyoxometalate (POM) as a homogeneous photocatalyst and compared with that of heterogeneous TiO$_2$ photocatalyst. Aqueous suspensions of the microorganisms (10^7–10^8 cfu ml^{-1}) and POM (or TiO$_2$) were irradiated with black light lamps. The POM-PCI was faster than (or comparable to) TiO$_2$-PCI under the experimental conditions employed in this study. The relative efficiency of POM-PCI was species-dependent. Among three POMs (H$_3$PW$_{12}$O$_{40}$, H$_3$PMo$_{12}$O$_{40}$, and H$_4$SiW$_{12}$O$_{40}$) tested in this study, the inactivation of *E. coli* was fastest with H$_4$SiW$_{12}$O$_{40}$ while that of *B. subtilis* was the most efficient with H$_3$PW$_{12}$O$_{40}$. Although the biocidal action of TiO$_2$ photocatalyst has been commonly ascribed to the role of photogenerated reactive oxygen species such as hydroxyl radicals and superoxides, the cell death mechanism with POM seems to be different from TiO$_2$-PCI. While TiO$_2$ caused the cell membrane disruption, POM did not induce the cell lysis. When methanol was added to the POM solution, not only the PCI of *E. coli* was enhanced (contrary to the case of TiO$_2$-PCI) but also the dark inactivation was observed. This was ascribed to the *in situ* production of formaldehyde from the oxidation of methanol. The interesting biocidal property of POM photocatalyst might be utilized as a potential disinfectant technology.
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1. Introduction

Homogeneous or heterogeneous photocatalysis plays a central role in many photochemical conversion processes. As for heterogeneous photocatalysis, semiconductor oxides including TiO$_2$ have been widely investigated for the complete oxidation of toxic contaminants in water and air (Ollis and Al-Ekabi, 1993; Hoffman et al., 1995; Choi, 2006). Since Matsunaga et al. (1985) reported the first application of TiO$_2$ photocatalysis to the inactivation of *Escherichia coli*, a number of studies on photocatalytic inactivation (PCI) of microorganisms have been conducted (Wei et al., 1994; Kikuchi et al., 1997; Cho et al., 2004,

2005). The PCI of biological cells can be similarly compared with the photocatalytic degradation of chemical compounds. The photo-induced radical chemistry involving reactive oxygen species drives not only the degradation of chemical compounds but also the inactivation or the death of microbial cells. It is generally believed that the hydroxyl radical, which is the major oxidant of TiO$_2$ photocatalysis, should attack and disrupt the cell wall or membrane to initiate the inactivation process (Ireland et al., 1993; Bekbölet, 1997; Lee et al., 1997; Cho et al., 2004).

Polyoxometalates (POMs) have been studied as a homogeneous photocatalyst (Maldotti et al., 1994; Weinstock, 1998; Androulaki et al., 2000; Hiskia et al., 2001a) and often similarly compared with its heterogeneous counterpart, TiO$_2$ (Kim et al., 2004; Park and Choi, 2005; Lv and Xu, 2006). POM is a well-organized metal–oxygen cluster anion, which initiates a variety of redox reactions

under UV-illuminated condition (Yamase, 1998; Hiskia et al., 2001b; Song and Barteau, 2004). Common POMs that have been investigated as photocatalysts include tungstosilicic acid ($H_4SiW_{12}O_{40}$), phosphotungstic acid ($H_3PW_{12}O_{40}$), and phosphomolybdic acid ($H_3PMo_{12}O_{40}$) (Hiskia et al., 2001a,b; Song and Barteau, 2004). POM and TiO_2 share the similar photochemical mechanisms in their operation. Photoexcited POMs carry a strong oxidant power to directly abstract an electron from substrates or to generate OH radicals through water oxidation as the bandgap excited TiO_2 does. Similarities between the homogeneous and heterogeneous photocatalysts (POMs vs TiO_2) have been recognized, and a few comparative studies on their photocatalytic behaviors were carried out (Ozer and Ferry, 2002; Kim et al., 2004; Park and Choi, 2005; Lv and Xu, 2006). Although the photocatalytic biocidal effects of TiO_2 have been widely recognized and investigated (Wei et al., 1994; Cho et al., 2004, 2005), the similar effects of POMs have not been reported yet. In the present work, a comparative study of POMs and TiO_2 as an inactivation photocatalyst was done using *E. coli* and *Bacillus subtilis* as a representative of Gram-negative and positive bacteria, respectively.

2. Experimental section

2.1. Chemicals and materials

$H_3PW_{12}O_{40}$ (Aldrich), $H_3PMo_{12}O_{40}$ (Fluka), and $H_4SiW_{12}O_{40}$ (Aldrich) were used as homogeneous photocatalysts without any further treatment. Each POM is abbreviated as PW_{12}, PMo_{12}, and SiW_{12}, respectively, throughout the text. TiO_2 (Degussa P25), a mixture of 80% anatase and 20% rutile with an average surface area of $50 \pm 15 \ m^2 \ g^{-1}$, was used as a heterogeneous photocatalyst. Methanol (MeOH; Samchun, Korea) was used as received. Deionized water was ultrapure (18 MΩ cm) and prepared by a Barnstead purification system. All glassware used in these experiments were washed with distilled water, and then autoclaved at 121 °C for 15 min. *E. coli* (ATCC 8739), a well-known indicator for Gram-negative bacterium, and *B. subtilis* (NRRL B-23049), a well-known indicator for Gram-positive bacterium, were chosen as the test microorganisms for PCI.

2.2. Photocatalytic inactivation experiments

POM (or TiO_2) was dissolved (or dispersed) in distilled water by simultaneous sonication and shaking for 30 s in an ultrasonic cleaning bath. *E. coli* and *B. subtilis* were grown in Luria Bertain-medium (Merck) containing $10 \ g \ l^{-1}$ tryptone, $5 \ g \ l^{-1}$ yeast extract, and $10 \ g \ l^{-1}$ NaCl at 37 °C with shaking at 200 rpm overnight. We used only harvested cells that were separated from the medium to avoid the interference of the LB-medium components in PCI reaction. *B. subtilis* that was used in this PCI test was cultured under the condition where the spore forma-

tion was not favored. The pH of TiO_2/*E. coli* suspension was adjusted to 7.1 using a phosphate buffer (KH_2PO_4/NaOH). In the absence of the phosphate buffer, microorganisms sampled from the TiO_2 suspension were not cultured well. It seems that the strong affinity between TiO_2 and microorganisms hinders the culturing process. Therefore, PCI experiments employing suspended TiO_2 particles were usually carried out in the phosphate buffer solutions (Matsunaga et al., 1985; Cho et al., 2004, 2005). On the other hand, when the pH of POM/*E. coli* suspension was adjusted to 7.1 using the same phosphate buffer used in the TiO_2-PCI experiment, no PCI of microorganisms was observed. The PCI activity of POM seems to work only at acidic condition since POMs are stable only at acidic conditions. Therefore, all POM-PCI experiments were conducted at acidic pH without using the phosphate buffer. POM-PCI and TiO_2-PCI experiments were carried out and compared at different pH because their optimal operating conditions were different. Incidentally, the pH effect on the TiO_2-PCI efficiency seems to be insignificant. A previous PCI study employing the same TiO_2 (P25 TiO_2 g l^{-1}) as a photocatalyst showed that the *E. coli* inactivation kinetics was not affected by pH (5.6, 7.1, and 8.2) at all (Cho et al., 2004).

Unlike TiO_2, POMs have the intrinsic biocidal effect even in the absence of light as Table 1 shows. The viability of *E. coli* under the dark environment was not inhibited by POM up to $[PW_{12}] = 0.7$ mM, $[SiW_{12}] = 0.1$ mM, and $[PMo_{12}] = 0.05$ mM. However, when [POM] increased above this critical value, *E. coli* was significantly inactivated even in the dark condition. Therefore, most PCI experiments in this work were conducted with [POM] at which the dark biocidal effect was not observed. The employed concentration of each POM was different: $[PW_{12}] = 0.35$, $[SiW_{12}] = 0.1$, and $[PMo_{12}] = 0.05$ mM. When we tried to compare the PCI activity of three POMs at the same POM concentration, some PCI activity was so high that the time profiles of the PCI could not be obtained. Therefore, the employed POM concentrations were the result of the adjustment so that the time profiles of $Log(N/N_0)$ in POM-PCI can be comparable in the same time scale (0–40 min) among different POMs and TiO_2.

Table 1
The dark inactivation of *E. coli* (in 20 min) in the presence of POMs (PW_{12}, SiW_{12}, PMo_{12})

SiW_{12}		PMo_{12}		PW_{12}	
[POM] (mM)	*E. coli* ($Log(N/N_0)$)	[POM] (mM)	*E. coli* ($Log(N/N_0)$)	[POM] (mM)	*E. coli* ($Log(N/N_0)$)
0	0	0	0	0	0
0.05	0	0.05	0	0.35	0
0.1	0	0.1	−2.7	0.5	0
0.25	−3.14	0.5	−4.3	0.7	0
0.3	−3.4	1	n.v.c.[a]	1	−3.11
0.5	−4.7			2	−3.21

N, concentration of microorganisms (in cfu per milliliter); N_0, initial N.
[a] No viable cells.