Photocatalytic bactericidal effect of silica gel-supported metalloporphyrin complexes

シリカゲル担持金属ポルフィリンの可視光殺菌効果

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Preface

This thesis entitled in "Photocatalytic bactericidal effect of silica-gel supported metalloporphyrin complexes (シリカゲル担持金属ポルフィリンの可視光殺菌効果)" has been accomplished by the author under the guidance by Professor Masahide Yasuda, Professor Haruhiko Yokoi, and Associate Professor Tsutomu Shiragami at the Department of Applied Chemistry, Faculty of Engineering, University of Miyazaki.

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Contents

General introduction

A study on "Photocatalytic bactericidal effect of silica gel-supported metalloporphyrin complexes" has been started under the following backgrounds.

Outbreak of bacteria: Recently, many mass media reported the bactericidal accidents occurred many places and times. Harmful microorganisms such as *Escherichia coli* O-157 or *Norovirus* set up group food poisoning[1]. Especially, *Legionella pneumophila* and the related bacteria have drawn much concern since the first outbreak of Legionnaires' disease in Philadelphia in 1976.[1] It has been reported that *L. pneumophila* coming from the many cooling towers, hot springs, and fountains caused Legionnaires' disease. In Japan, also, the large outbreaks of Legionnaires' disease occurred that 7 persons killed and 295 persons passed Legionnaires' disease in the public hot spring which is located in Hyuga city, Miyazaki Prefecture, Japan (Scheme 1).[2] Chemical sterilization using sodium hypochlorite is a general method to kill these hazardous microorganisms. However, it causes loss of hot spring taste, harmful act for human bodies, and environmental pollution.

2002年(平成14年)8月9日　金曜日　宮崎日日新聞

レジオネラ菌

源水タンクで大量発生か

日向市見解「温泉水排出せず」

日向市幸脇の第三セクター・日向サンパーク温泉「お舟出の湯」で発生したレジオネラ症集団感染で、同市は八日、記者会見を行い、泉源からくみ上げた温泉水を源水タンク（容量三十ﾄﾝ）内に滞留させたまま排出していなかったため、レジオネラ菌が大量発生した可能性があるとの見解を示した。

日向市によると、温泉水は今年六月七日からタンクに貯水開始。同十二、十三日には設備検査で浴槽に湯を張った。その後、施工業者がタンクの排出バルブを閉めた状態にしていたため、十八日まで約二十五ﾄﾝの湯が滞留した。

さらにプレオープン（二十、二十一日）後も、二十一日から二十八日までタンク内の湯を排出していなかった。施設の開業後、この湯の一部は加熱した上、浴槽で使用されたという。

県保健業務課によると、開業前の六月中に入浴した利用者からも感染・疑似感染者数人が発生。県が十九日に実施し二十五日に報告した施設の水質検査では、浴槽のほか、源水タンクとつながった温泉スタンドでも基準を上回るレジオネラ菌を検出している。このため、市では温泉水が滞留していたタンクがレジオネラ菌に汚染されていたとみている。

一方、施設側が七月二十六日に独自に実施した水質検査では、検査地点六カ所すべてでレジオネラ菌が検出されなかったことを明らかにした。

施設では県の指導を受けて自主検査前日の二十四、二十五日に源水タンク、配管、浴槽、ろ過機を通常より強い塩素で洗浄していた。市は「当初から、それをやっていれば（集団感染はなかった）」との認識を示した。

施設は昨年一月着工。同十月には厚生労働省のレジオネラ症対策マニュアルに基づき、計画を一部変更、今年三月に完成している。その後、施設側と業者は管理契約を結んでいなかった。

記者会見でレジオネラ菌発生について見解を示す市幹部＝8日午後、日向市役所

「なぜこんな目に」

悔しさにじます遺族

「健康増進のために行っただけなのに…」。日向サンパーク温泉「お舟出の湯」で発生したレジオネラ集団感染で、八日に亡くなった同市内の七十代男性の遺族は、やりきれない表情で悔しさをにじませた。男性は入院後、少しずつだが快方に向かっていただけに、男性の妻([illegible])ら家族は「なぜこんな目に」とショックを隠せないでいる。

親族の男性([illegible])の話では、亡くなった男性が温泉に入ったのは一度きりだった。数日後には発熱し、けん怠感を訴えた。その翌日の十四日、肺炎症状から呼吸困難に陥り病院に運ばれた。搬送後すぐに人工呼吸のための管がのどに通され、闘病生活に。親族以外は面会謝絶の状態が続いた。

二度訪れた危篤状態も乗り切った。六日までの数日間、人工呼吸器のレベルを下げて自呼吸のリハビリを行えるまでになっていた矢先だった。

七日午前には肺炎を起こし下血するなど容態が急変。同日午後には輸血を施したが、家族らの願いは届かなかった。

親族男性は「（施設への）怒りというより、今は悲しみの方が大きい」と肩を落とした。

Scheme 1. Outbreak of Legionnaires' disease in hot spring facility in Hyuga city, Miyazaki pref, Japan (Miyazaki Nichinichi Shinbun on Aug. 9th, 2002).

Photocatalysts: Much attention has been paid to photocatalysts as an environmentally friendly process to degrade organic compounds in water and air. Among the photocatalysts, it is well known that UV light irradiation of powdered TiO_2 in aqueous solution generates a hydroxyl radical which works as a strong oxidizing agent.[3-9] Moreover it has been reported that powdered TiO_2 is applicable for the sterilization of various microorganisms such as *Lactobacillus acidophilus*, *Saccharomyces cerevisiae*, *E.*

coli and *Chlorella vulgaris* under irradiation by UV light.[10-13] However, UV light used for activation of the TiO_2 is harmful to humans. Therefore, a photocatalyst operating under irradiation by visible light such as sunlight or a fluorescent lamp is a desirable development.

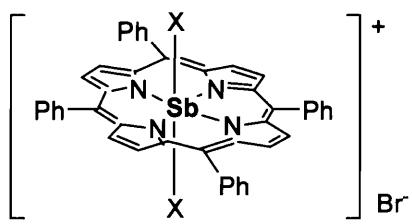
Visible light-driven photocatalyst: Metalloporphyrins that have strong absorption in the visible region have received much attention as visible light-driven photocatalyst.[14-17] However, metalloporphyrins are insoluble in aqueous solution. Therefore, Yasuda and his co-workers have immobilized dihydroxo(tetraphenylporphyrinato)antimony(V) (SbTPP) on silica gel and have found that the silica gel-supported SbTPP (SbTPP/SiO_2) was effective for the photochemical dechlorination of 4-chlorophenol[18] and the photooxidation of simple alkenes under visible light irradiation.[16,19-20]

Silica gel: As mentioned above, metalloporphyrins are insoluble in aqueous solution. Therefore, it is requisite to immobilize metalloporphyrins on the carrier. Silica gel (SiO_2) is safe and stable carrier, and has its high transparency of visible light, wide surface area, and the ability to adsorb a variety of materials. Therefore, it is expected that the new types of photocatalysts are developed by the combination of metalloporphyrins with SiO_2.

Accordingly, it is requisite to develop the photocatalytic bactericidal technique

using the visible light-driven catalyst. The SbTPP and its phosphorus analogs (Scheme 2) are expected to be suited as visible light driven photo catalyst for photo bactericidal materials.

In this thesis, the author will investigate the preparation of visible light-driven photocatalyst which consists of metalloporphyrin silica gel-support and will produce the apparatus and module for bactericidal experiments. Using these photocatalyst and apparatus, the author will elucidate the bactericidal activity of the metalloporphyrin catalyst against *E. coli* and *Legionella* species.



X= Br, Cl, OH, OR

SbTPP

Scheme 2. The chemical structure of dihydroxo(tetraphenylporphyrinato)antimony(V) (SbTPP).

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Chapter 1.

Bactericidal effect of a silica gel-supported porphyrinatoantimony(V) complex under visible light irradiation

1-1. Introduction

As mentioned in General Introduction, it is well known that the tetraphenylporphrinatoantimony(V) complex (SbTPP) can sensitize the photooxidation of simple alkenes with higher oxidation potentials under visible light irradiation.[1-2] Furthermore, it has been found that the SiO_2-supported SbTPP photocatalyst (SbTPP/SiO_2) was effective for the photochemical dechlorination of 4-chlorophenol.[3] Because SbTPP has the high oxidation ability, therefore, it is expected that the photocatalyst might have ability to inactivate microorganisms. In this chapter, the bactericidal activity and inactivation properties of SbTPP/SiO_2 under irradiation by visible light will be elucidated.

1-2. Experimental section

Preparation of photocatalyst

Dihydroxo(tetraphenylporphyrinato)antimony(V) bromide, [SbTPP(OH)$_2$]Br, was prepared according to the literature.[1-2, 4] Into the toluene solution (400 cm^3) of [SbTPP(OH)$_2$]Br (170 mg), silica gel powder (p-SiO_2, 300 mesh, 40 μmϕ, 30 g, BW300, Fuji Silysia Chemical Ltd., Japan) was added and the mixture was refluxed for 8 h.[3] The treated silica gel was filtered and then dried under reduced pressure to give the silica gel powder-supported [SbTPP(OH)$_2$]$^+$ photocatalyst (SbTPP/p-SiO_2), where the content of the SbTPP(OH)$_2^+$chromophore was 0.87 wt%. Into a MeOH–toluene solution (1:4, v/v, 500 cm^3) of [SbTPP(OH)$_2$]Br (60 mg), silica gel beads (b-SiO_2, 1.7–4.0 mmϕ, 70 g, CARIACT Q-10, Fuji Silysia Chemical Ltd.) was added followed by standing for 18 h. The MeOH was evaporated from the solution at 40 °C under reduced pressure. The treated silica gel was filtered and then dried under reduced pressure at 40 °C to give the b-SiO_2-supported [SbTPP(OH)$_2$]$^+$ photocatalyst (SbTPP/b-SiO_2) where the content of SbTPP(OH) $_2^+$ chromophore was 0.087 wt%. Characteristics of SbTPP/p-SiO_2 and SbTPP/b-SiO_2 are shown in Table 1-1.

Table 1-1. Characterization of the photocatalyst ($SbTPP/SiO_2$)[a)]

Characteristics	$SbTPP/p\text{-}SiO_2$	$SbTPP/b\text{-}SiO_2$
Average particle size (mm)	0.04	3.0
Content of $[SbTPP(OH)_2]$ Br (wt%)	0.87	0.087

a) Antimony eluted from the photocatalyst was <0.005 mg·dm^{-3} under the treatment at 50 °C for 72 h in aqueous solution.

Preparation of cell suspension

Escherichia coli K-12 (IFO3301) was used for bactericidal experiments. The bacterium was cultured aerobically at 30 °C for 8 h in a basal medium (pH 6.5) consisting of Bactotryptone (Difco) (10 g·dm^{-3}), yeast extract (5 g·dm^{-3}), and NaCl (10 g·dm^{-3}). After centrifugation of the cultured broth for 10 min, the harvested cells were washed with physiological saline NaCl (7 g·dm^{-3}) and then suspended in the saline to give a cell suspension of *E. coli* (6.4×10^4 cells·cm^{-3}).

Photocatalytic reaction in L-type glass tube

A reaction mixture containing the suspended $SbTPP/p\text{-}SiO_2$ (usually, 10 mg), 1.0 cm^3 of the cell suspension of *E.coli*, and 9.0 cm^3 of phosphate buffer (100 mmol·dm^{-3} pH 7.0) was poured into an L-type glass tube (length 18 cm, diameter 1.5 cm). The L-type glass tube was set on a reciprocal shaker and irradiated by two

fluorescent lamps, 15 W each, which were set above the shaker. Reaction temperature was kept constant at 30 °C.

Photocatalytic reaction in an oval-type photoreaction apparatus

An oval-type photoreaction apparatus which consisted of a fluorescent lamp (18 W), a column reactor (2 cmϕ×50 cm, 150 cm^3) packed by the SbTPP/b-SiO_2 (80 g), and a holder (500 cm^3) was used (Figure 1-1). In the apparatus, the visible light emitted from a fluorescent lamp set on one focus point of an oval mirror was intended to be concentrated on the reactor set at another focus point. Irradiation was performed at ambient temperature for the cell suspension fed from the holder at the flow rate of 20 $cm^3 \cdot min^{-1}$ and the treated cell suspension was returned to the holder.

Determination of viable cell numbers

The number of viable cells in *E. coli* suspensions was determined by plating the cell suspensions on the basal medium supplemented with agar (20 $g \cdot cm^{-3}$) after suitable dilutions. Then the colonies which appeared after 14 h incubation at 30 °C were countered for three replicate plates.

Measurement of light intensity

Light intensity on the surface of the L-type glass tube was determined by a power meter (TQ8210, Advantest Ltd., Japan) at the wavelength of 420 nm.

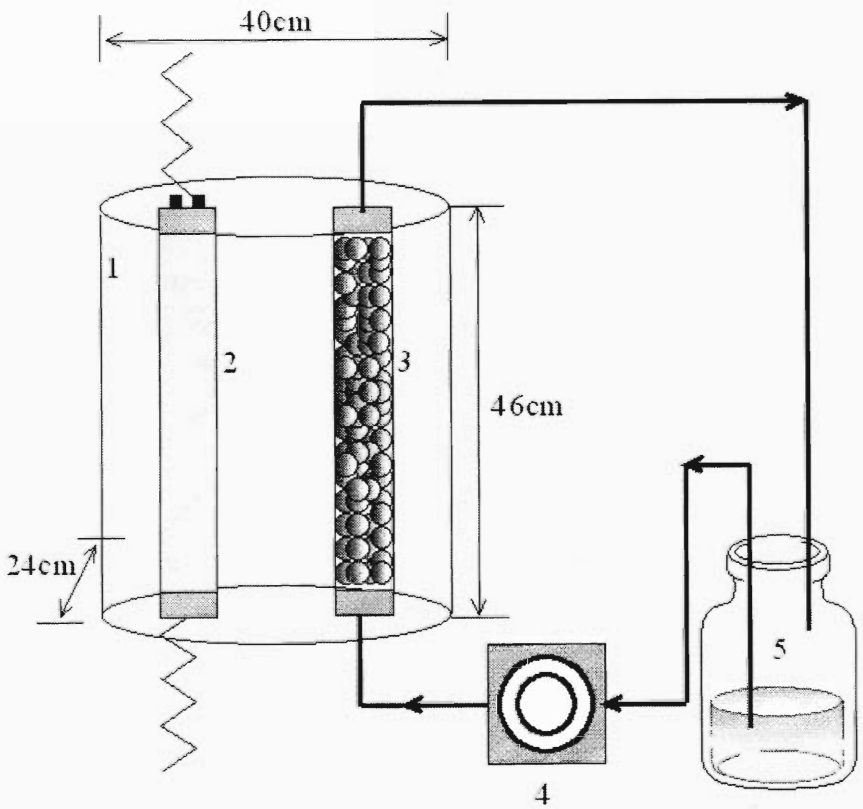

Figure 1-1. Schematic diagram of oval-type photoreaction apparatus: (1) oval-type mirror; (2) fluorescent lamp (18 W); (3) column reactor packed by photocatalyst; (4) peristaltic pump; (5) sample holder.

1-3. Results and discussion

Photocatalytic inactivation in L-type glass tube

Figure 1-2 shows the time courses of survival ratio of *E. coli* when the cell suspensions were irradiated by a fluorescent lamp (21 $W \cdot m^{-2}$) in the presence of SbTPP/p-SiO_2 photocatalyst (1 $g \cdot dm^{-3}$). Upon irradiation for 60 min, the viable count of *E. coli* decreased from the initial concentration (6.4×10^3 cells$\cdot cm^{-3}$) to about 40 cells$\cdot cm^{-3}$ and its survival ratio was 0.6%. As control experiments, irradiation in the absence of the photocatalyst and dark reaction in the presence of the photocatalyst were also performed. Each run left the initial viable *E. coli* unchanged, showing that the SbTPP/p-SiO_2 photocatalyst has bactericidal activity only under irradiation conditions.

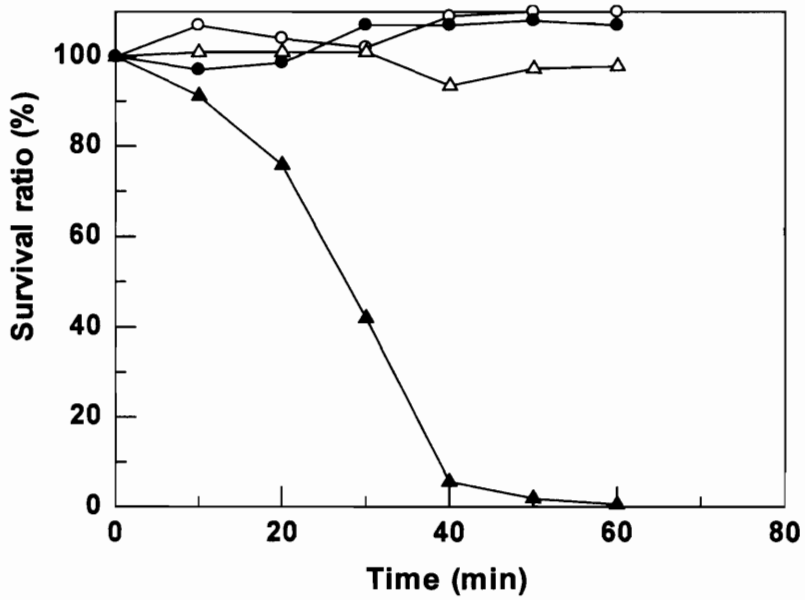

Figure 1-2. Bactericidal effect of SbTPP/p-SiO_2 on *E. coli* under irradiation by fluorescent lamp. Initial concentration of the bacterial cells and SbTPP/p-SiO_2 concentration in reaction mixtures were 6.4×10^3 cells·cm^{-3} and 1 g·dm^{-3}, respectively. Light intensity was 21 W·m^{-2}. (○) Dark reaction in the absence of the photocatalyst; (●) under irradiation in the absence of the photocatalyst; (△) dark reaction in the presence of the photocatalyst; (▲) under irradiation in the presence of the photocatalyst.

It has been reported that UV light irradiation of TiO_2 powders (1 g·dm^{-3}) for 30–60 min has achieved the inactivation of *E. coli*.[5-7] In order to compare the abilities of the present SbTPP/p-SiO_2 photocatalyst with TiO_2 photocatalyst under the visible light irradiation, the photocatalytic inactivation of the bacterial cells using a few types of commercially available TiO_2 was also carried out. Anatase-type TiO_2 (Wako Pure Chemicals Ltd., Japan), rutile-type TiO_2 (Wako Pure Chemicals Ltd., Japan) and TiO_2 supported on silica-gel powder (type HQA51, Shinto V-cerax Ltd., Japan) were used. After visible light irradiation for 120 min, the survival ratios of *E. coli* were more than

104, 98, and 85% for the cases of anatase-type and rutile-type TiO_2, and TiO_2 supported on silica-gel powder (1 $g \cdot dm^{-3}$), respectively. Therefore, it was confirmed that the SbTPP/p-SiO_2 was much superior to TiO_2 in bactericidal activity under irradiation by visible light.

The influence of SbTPP/p-SiO_2 dose on the bactericidal reaction of *E. coli* cells is shown in Figure 1-3 where the concentration of the SbTPP/p-SiO_2 was varied in the range 0.1–5 $g \cdot dm^{-3}$. Initial concentration of the bacterial cells and light intensity were 6.2×10^3 cells$\cdot cm^{-3}$ and 21 $W \cdot m^{-2}$, respectively. Although bactericidal activity at 0.1 $g \cdot dm^{-3}$ of SbTPP/p-SiO_2 was low, significant inactivation of the bacterial cells was observed at a concentration of more than 0.5 $g \cdot dm^{-3}$ of SbTPP/p-SiO_2, showing that the cell viability decreased with an increase in the SbTPP/p-SiO_2 dose. Consequently, the optimized concentration of the SbTPP/p-SiO_2 under visible light irradiation was nearly equal to 1 $g \cdot dm^{-3}$ which was the reported TiO_2 concentration for the maximum inactivation of *E. coli*[6] and *Streptococcus sorbinus*.[8]

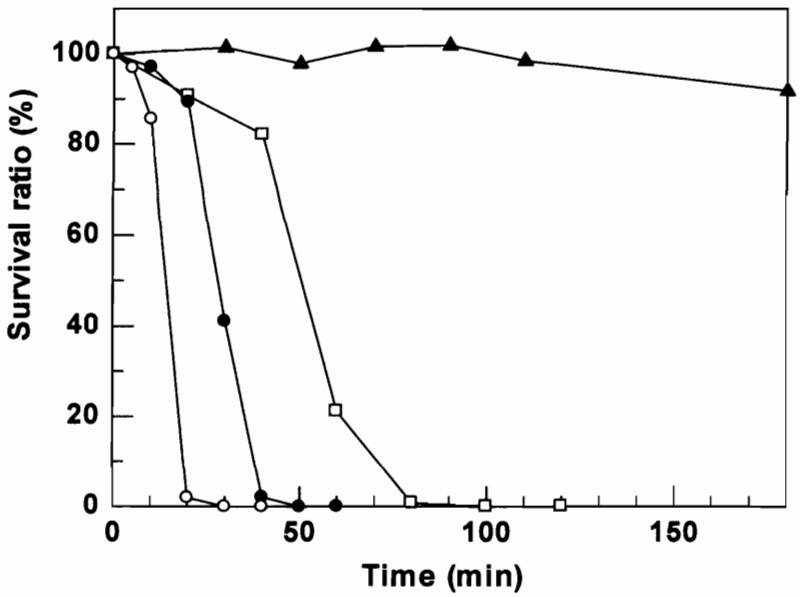

Figure 1-3. Effect of SbTPP/p-SiO_2 concentration in reaction mixture on bactericidal reaction of *E. coli* under irradiation by fluorescent lamp. Initial concentration of the bacterial cells and light intensity were 6.2×10^3 cells·cm^{-3} and 21 W·m^{-2}, respectively. (○) 5 g·dm^{-3}; (●) 1 g·dm^{-3}; (□) 0.5 g·dm^{-3}; (▲) 0.1 g·dm^{-3}.

The effect of light intensity on the bactericidal activity was investigated in a reaction mixture containing 6.4×10^3 cells·cm^{-3} of *E. coli* and 1 g·dm^{-3} of SbTPP/p-SiO_2 (Figure 1-4). The rate of cell inactivation increased with an increase in light intensity from 7 to 26 W·m^{-2}. Figure 1-5 shows the effects of the initial concentration of *E. coli* on the time courses of viable cell numbers where initial cell concentrations varied from 2.8×10^3 to 2.3×10^4 cells·dm^{-3}. Concentration of SbTPP/p-SiO_2 and light intensity were 1 g·dm^{-3} and 21 W·m^{-2}, respectively. Inactivation rate of the bacterial cells increased with increasing initial cell concentration. Half-life time of the bacterial cells was around 18 min, independently of the initial cell concentration.

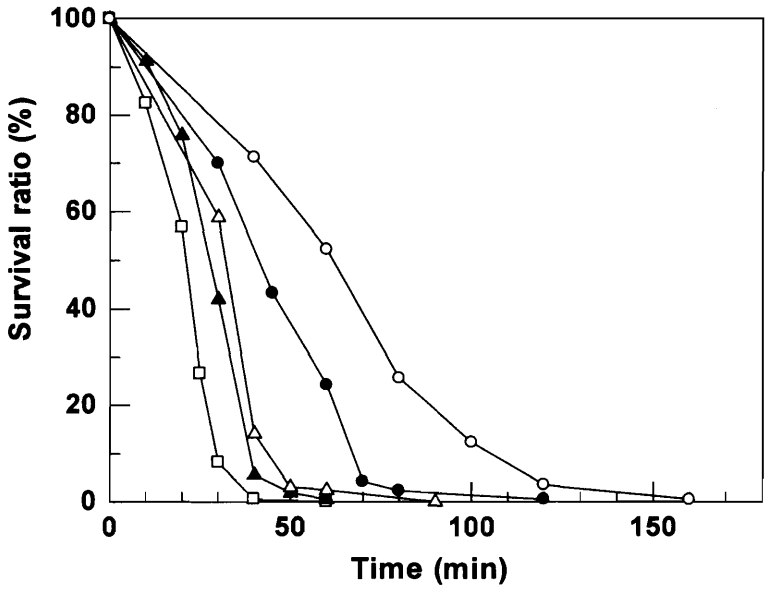

Figure 1-4. Effect of light intensities emitted with fluorescent lamp on bactericidal reaction of *E. coli* by SbTPP/p-SiO_2. Initial concentration of the bacterial cells and SbTPP/p-SiO_2 concentration in reaction mixtures were 6.4×10^3 cells·dm^{-3} and 1 g·dm^{-3}, respectively. (O) 7 W·m^{-2}; (●) 11 W·m^{-2}; (△) 17 W·m^{-2}; (▲) 21 W·m^{-2}; (□) 26 W·m^{-2}.

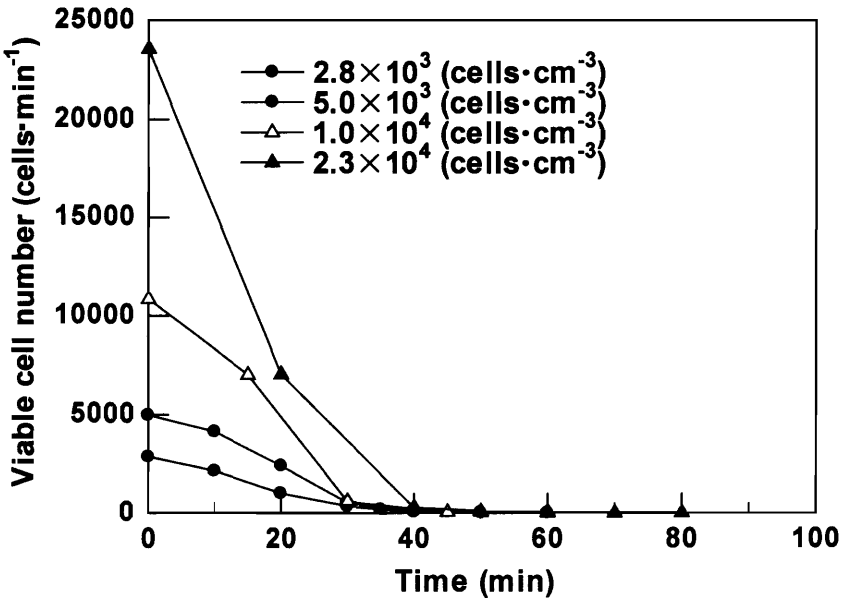

Figure 1-5. Influence of initial concentration of *E. coli* cells on bactericidal reaction by SbTPP/p-SiO_2 under irradiation with fluorescent lamp. SbTPP/p-SiO_2 concentration in reaction mixture and light intensity were 1 g·$dm^{-3}$and 21 W·m^{-2}, respectively. (O) 2.8 $\times10^3$ cells·cm^{-3}; (●) 5.0×10^3 cells·cm^{-3}; (△) 1.0 $\times10^4$ cells·cm^{-3}; (▲) 2.3×10^4 cells·cm^{-3}.

The bactericidal activity of SbTPP/p-SiO_2 for *E. coli* cells under irradiation of sunlight was also examined. The L-type glass tubes containing 6.4×10^3 cells·cm^{-3} of bacterial cells, 0.5 g·dm^{-3} of SbTPP/p-SiO_2 or 0.5 g·dm^{-3} of TiO_2 supported on silica-gel powder were set on the reciprocal shaker, then put on the roof of a building and exposed to sunlight for 60 min. Light intensity varied from 200 to 1100 W·m^{-2} and average light intensity was about 530 W·m^{-2}. Temperature of reaction mixtures varied from 28 to 32 °C and average temperature was 30 °C. After 10min irradiation, viable cells were not detected in the case of the SbTPP/p-SiO_2, but almost all the bacterial cells were alive in the cases of TiO_2 or without photocatalyst, as shown in Table 1-2. Therefore, it was confirmed that SbTPP/p-SiO_2 under sunlight has high ability to inactivate bacterial cells.

Incident light was absorbed exclusively by SbTPP/p-SiO_2. However, the excited state of SbTPP/p-SiO_2 was too short-lived to make contact directly with the bacteria. Therefore, it is suggested that the reactive species generated by irradiation of the photocatalyst is responsible for the bactericidal activities. As a candidate for the reactive species, singlet oxygen was proposed, since it is relatively long-lived. It is suggested that the energy transfer from the excited triplet state of SbTPP/p-SiO_2 (triplet energy : 1.63 eV) to molecular oxygen (triplet energy : 0.98 eV) was responsible for the

initiation process.[9] The singlet oxygen ($^1O_2$) generated by energy transfer from SbTPP/p-SiO_2 to O_2 would induce the fatal damage of the bacteria. The present mechanism is different from the mechanism proposed for the TiO_2-photocatalysation, which is thought to involve reaction with OH radicals.

Table 1-2. Bactericidal effect of SbTPP/p-SiO_2 under irradiation of sunlight [a)]

Time (min)	Survival ratio (%)		
	SbTPP/p-SiO_2	TiO_2	Blank
0	100	100	100
10	0	103	99
30	0	83	90
60	0	63	79

a) Light intensity of sunlight varied from 200 to 1100 $W \cdot m^{-2}$ during 60 min and average intensity was about 530 $W \cdot m^{-2}$. Average temperature of reaction mixtures was about 30 °C

Photocatalytic inactivation in oval-type photoreaction apparatus

Since an L-type test tube is too small to apply for inactivation in practice, an oval-type photoreaction apparatus consisting of a column reactor packed with SbTPP/p-SiO_2, a fluorescent lamp and a holder was developed and used for the continuous bactericidal reaction. Cell suspension (500 cm^3) containing *E. coli* (4.5×10^4 cells$\cdot cm^{-3}$) in 100 mmol$\cdot dm^{-3}$ phosphate buffer (pH 7.0) was circulated in the apparatus and the viable cell number in the holder was counted. As shown in Figure

1-6, by keeping under dark conditions for 10 h, the concentration of *E. coli* cells decreased gradually until the concentration had reached a stable concentration of 2.0×10^4 cells·cm^{-3}. It seems that the decrease of cell number was caused by the adsorption of the bacterial cells onto the surface of SbTPP/p-SiO_2. Then, photocatalytic inactivation was started under irradiation. Viable cell number in the cell suspension decreased with increase of irradiation time and the bacterial cells were entirely inactivated after irradiation for 16 h, indicating that a column reactor packed by the SbTPP/p-SiO_2 is applicable to inactivation of water contaminated with bacterial cells. It seems that incident light is mostly absorbed on the photocatalyst located on the outer layers of the column, and bactericidal activity in the outer area is higher than that in central area. The oval type photoreaction apparatus is effective for the bactericidal reaction since the surface of the column reactor is equally irradiated by fluorescence light with an oval mirror. Moreover, it is considered that the photoreactor with the photocatalyst driven by visible light is advantageous for inactivation of bacterial cells in water, since the transmittance of visible light in water is very high compared with UV light used for TiO_2.

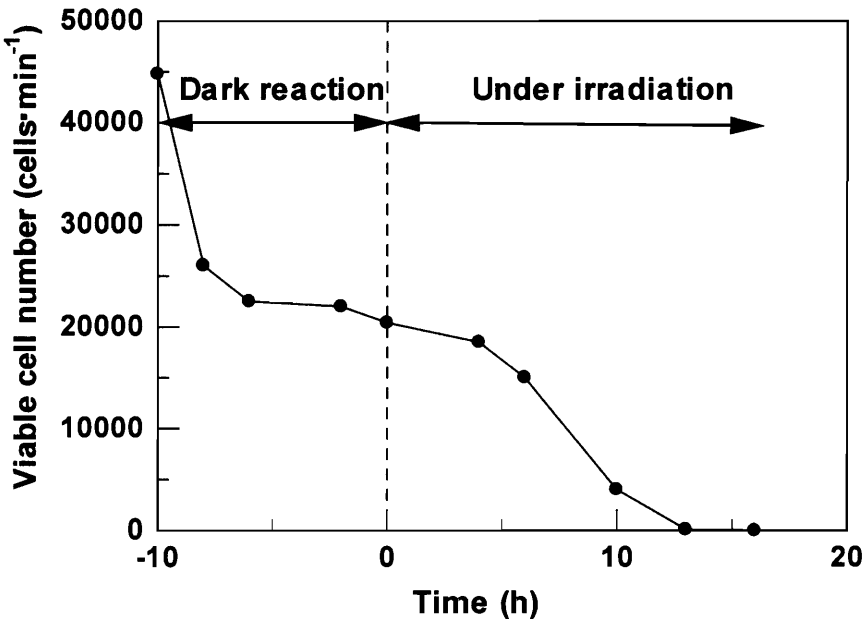

Figure 1-6. Bactericidal action for *E. coli* with SbTPP/b-SiO_2 using oval type photoreaction apparatus.

1-4. Conclusions

Thus, we found that SbTPP/SiO_2 could inactivate *E. coli* cells by irradiation with fluorescent lamp and sunlight. There have been no reports about the photocatalyst showing high bactericidal activity under irradiation by visible light. We propose an environmentally friendly process using the SbTPP/SiO_2 photocatalyst under visible light irradiation which will be widely applicable to the inactivation of microorganisms in many areas.

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Chapter 2.

Visible-light bactericidal effect of silica gel-supported porphyrinatoantimony(V) catalyst on *Legionella* species occurring in the living environmental fields

2-1. Introduction

In chapter 1, the bactericidal effect of $SbTPP/SiO_2$ catalyst on *E. coli* has been elucidated. Recently, large outbreaks of Legionnaires` disease have been reported in Portugal, Netherlands, and Spain[1-4]. The natural habitats for *L. pneumophila* are a wide range of aquatic bodies including lakes, streams, and artificially constructed aquatic reservoirs such as fountains and cooling towers. In this chapter, therefore, the author will report on the photocatalytic bactericidal activity of the $SbTPP/SiO_2$ to reduce *Legionella* concentrations and our practical experiments involving a cooling tower and public fountain which can be found in our living environment.

2-2. Experimental section

Instruments

For the elemental analysis of the catalysts, the catalysts (2 g) were calcined at 950 °C for 2 h and put into a mixture of water (2 cm^3) and HF solution (46%, 10 cm^3) and heated at 200 °C to remove Si component as SiF_4. The residual components were dissolved in hot HNO_3 (1 $mol \cdot dm^{-3}$, 5 cm^3) and subjected to the elemental analysis of Al and Sb on a Shimadzu AA-6200 atomic absorption spectrometer and a Shimadzu ICPS-7500 inductively coupled plasma (ICP) spectrometer, respectively. The atomic absorption analysis of other metals such as Na, Ca, Fe and Mg metals was performed after the addition of aqueous HCl solution of La_2O_3 (5 wt%) as the matrix modifier. Microspectroscopic analysis of the catalysts was performed on an Olympus FV-300 confocal laser scanning microscope (CLSM) equipped with spectrophotometer (STFL 250, Seki Technotron) linked to CLSM with an optical fiber. Light intensity was measured at the wavelength of 420 nm by a power meter (TQ8210, Advantest).

Preparation of the photocatalyst

$[SbTPP(OH)_2]Br$ was prepared according to the literature.[5-7] Into toluene solution (400 cm^3) of $[SbTPP(OH)_2]Br$ (170 mg), silica-gel powder (p-SiO_2; 30 g, 300

mesh, 0.04 mmϕ, 429 $m^2 \cdot g^{-1}$, BW300, Fuji Silysia Chemical Ltd., Japan) was added and then refluxed for 18 h.[8] The treated silica-gel was filtered, washed with acetone (100 cm^3), and then dried under reduced pressure to give the p-SiO_2-supported $[SbTPP(OH)_2]^+$ catalyst (SbTPP/p-SiO_2) where the content of $[SbTPP(OH)_2]^+Br^-$ chromophore was 0.87 wt% (Figure 2-1).

Into MeOH-toluene solution (1:4 v/v, 500 cm^3) of $[SbTPP(OH)_2]Br$ (35 mg), silica gel beads (b-SiO_2; 70 g, 1.7-4.0 mmϕ, 306 $m^2 \cdot g^{-1}$, CARIACT Q-10, Fuji Silysia Chemical Ltd. Japan) were added and then the solution was made to stand for 18 h. The MeOH was removed by distillation from the solution. The treated silica gel was filtered, dried at 70 °C, washed with acetone (200 cm^3), and then dried at 70 °C to give the b-SiO_2-supported $[SbTPP(OH)_2]^+$ catalyst (SbTPP/b-SiO_2) where the content of $[SbTPP(OH)_2]^+Br^-$ chromophore was 0.05 wt%. The prepared catalyst was identified by the observation of the characteristic fluorescence of SbTPP chromophore by CLSM analysis (Figure 2-2).

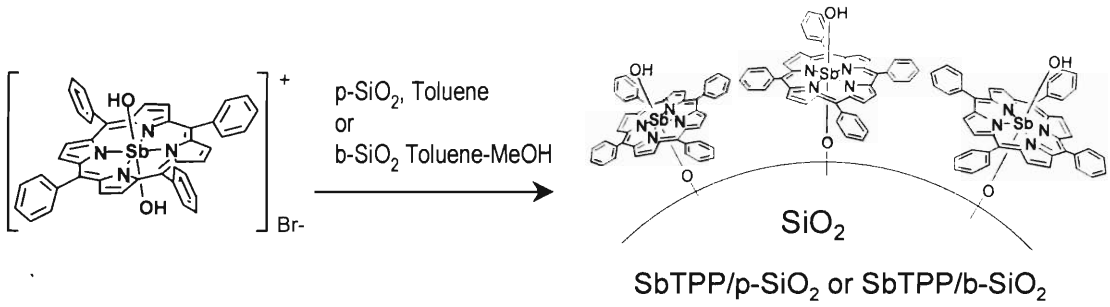

Figure 2-1. Preparation of SbTPP/p- or b-SiO_2 from the reaction of $[SbTPP(OH)_2]Br$ with SiO_2.

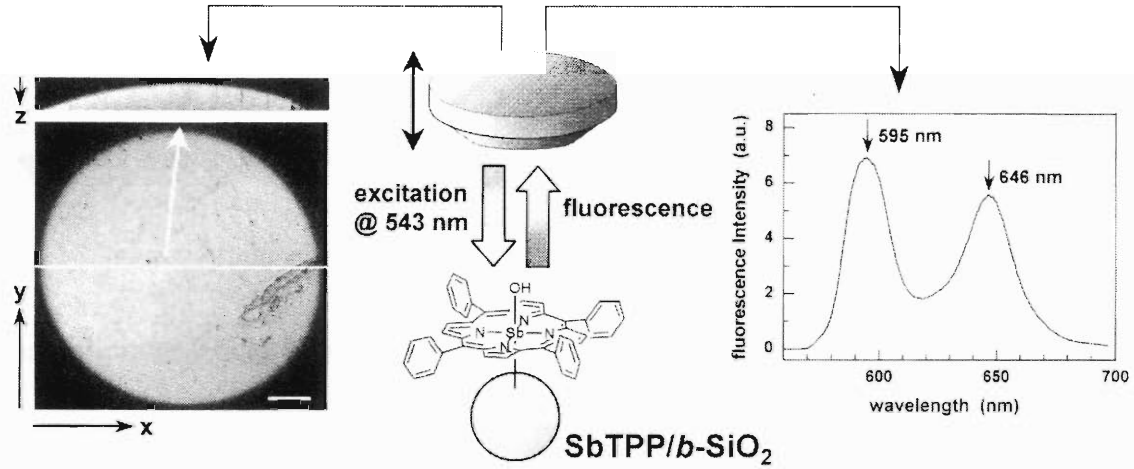

Figure 2-2. The imaging and fluorescence spectra of SbTPP/b-SiO_2 measured on CLSM.

Photocatalytic sterilization in L-type glass tubes

The bactericidal activities of the SbTPP/p-SiO_2 on *L. pneumophila* were examined in an L-type glass tube in similar manner to the method reported for *E. coli* in chapter 1. *L. pneumophila* was isolated from a cooling tower and identified to be Type I by the PCR method using a leg primer. The phosphate buffer (9 cm^3, 100 $mmol \cdot dm^{-3}$, pH 7.0), the cell suspension of *L. pneumophila* (1.0 cm^3, ca. 10^6 CFU/100 cm^3 ; CFU = Colony Forming Unit), and SbTPP/p-SiO_2 (10 mg) were poured into the L-type glass

tube (length 18 cm, diameter 1.5 cm). The L-type glass tube was set on a reciprocal shaker and irradiated by two fluorescent lamps, 15 W each, which were set above the shaker. Light intensity on the surface of the L-type glass tubes was 21 $W \cdot m^{-2}$ and the reaction temperature was kept constant at 30 °C.

A portion (0.1 cm^3) of the reaction mixture was directly plated on a selective medium for *Legionella* species: WYO α agar medium (Eiken Chemicals Co., Ltd, Japan) consisting of 3 g of glycine, 5 mg of vancomycin, 10^6 IU of polymixin B, and 80 mg of amphotericin B. The colonies of *L. pneumophila*, which appeared after incubation for 7 days at 36 °C were counted in three replicate plates.

Practical experiments in a cooling-tower using a cylindrical bactericidal apparatus

The bactericidal experiment was performed in a cooling tower which was set in a hospital in Miyazaki city, using a cylindrical apparatus (20 cmϕ × 50 cm, Figure. 2-3A) consisting of 7 fluorescent lamps (18 W, 4.3 cmϕ × 50 cm) and the SbTPP/b-SiO_2 catalyst (4.0 kg), as shown in Figure 2-3B. Water in the holder (800 dm^3) of the cooling tower was fed to the cylindrical vessel by a pump at 28 $dm^3 \cdot min^{-1}$ and then the treated water was returned to the holder. The average retention time was calculated to be 26 sec. In the cylindrical vessel, the SbTPP/b-SiO_2 catalyst was irradiated by

visible-light emitted from fluorescent lamps at ambient temperature. The sampling was carried out at the outlet of the apparatus at 3-7 day intervals. At same time, the atmospheric temperatures were recorded as the average values of the highest temperature of Miyazaki city during three days of the sampling day and two days before the sampling day. Viable cell numbers of bacteria that were mostly presumed to be *Legionella* species were determined as follows. The sample water (1000 cm^3) was filtrated by membrane filter (0.45 μm HA, Millipore) under reduced pressure. Into the vessel (100 cm^3) containing the microbes adhering to the membrane filter, an aqueous solution (5 cm^3) was added and then the vessel was shaken vigorously. Additional aqueous saturated KCl solution (5 cm^3; pH 2.2) containing 0.2 mol dm^{-3} HCl was added to the vessel and which then was shaken and made to stand exactly for 20 min at room temperature to give the prepared solution. A portion (0.1 cm^3) of the prepared solution was plated on a WYO α agar medium and incubated for 7 days at 36 °C. Wet, smooth, and bluish-white colonies were counted on three replicate plates.

Practical experiments in a fountain using a leaf-type bactericidal apparatus

Practical experiments were performed in a public fountain that was filled with 13 m^3 of water in a period from November 8, 2002 to October 29, 2003 in Miyazaki

City. Bactericidal effects were examined using a leaf-type photocatalytic bactericidal apparatus (20 cmϕ × 2 cm; Figure 2-3C) containing the SbTPP/b-SiO_2 catalyst (80 g) under sunlight irradiation, as shown in Figure 2-3D. The samplings were arbitrarily carried out at the pool of the fountain at 5-7 day intervals. At same time, the atmospheric temperatures were recorded as mentioned above. Determination of viable cell numbers of *Legionella* species was carried out in the same manner as in the practical experiments in the cooling-tower.

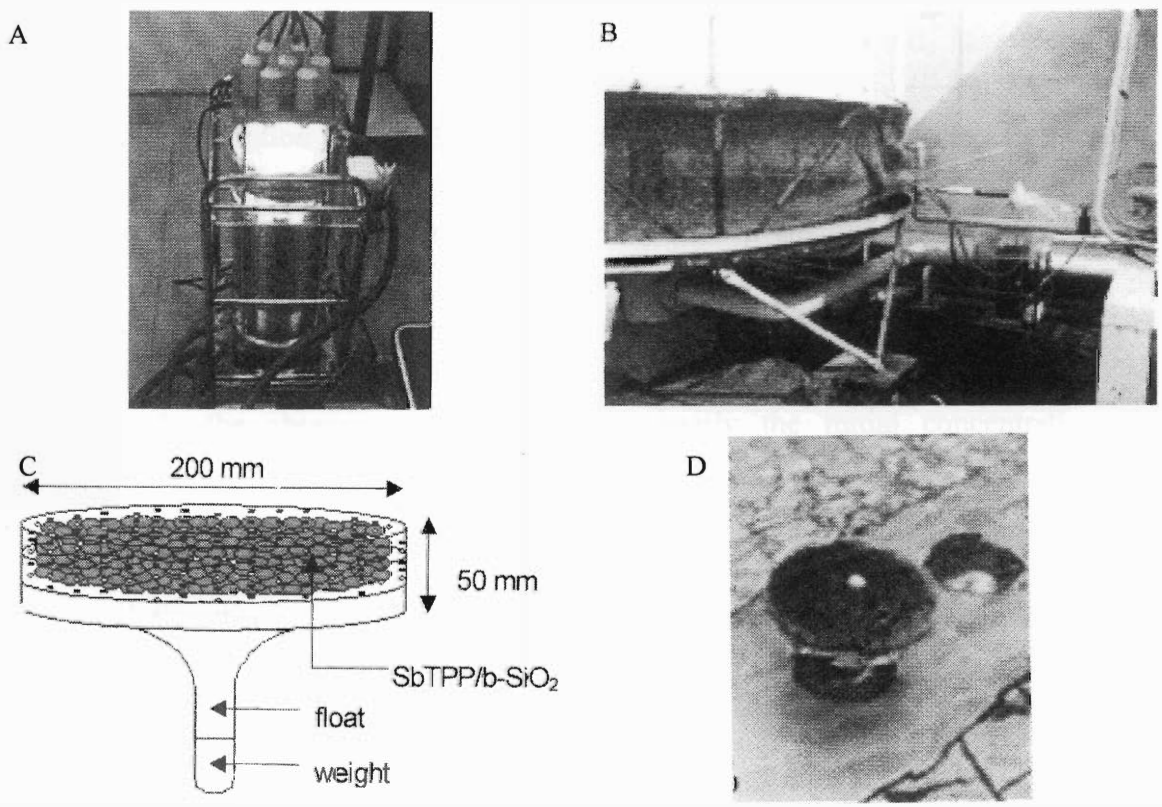

Figure 2-3. A cylindrical type photo-bactericidal apparatus (A) was set in the cooling-tower (B). A leaf-type photo-bactericidal apparatus (C) was installed in the fountain (D).

2-3. Results and discussion

Photocatalytic sterilization in L-type glass tube

The bactericidal effect of SbTPP/SiO_2 on *Legionella* species in L-type glass tubes was carried out under irradiation by fluorescent lamps. Figure 2-4 shows time courses of survival ratio of *Legionella* species in L-type glass tubes where the SbTPP/p-SiO_2 photocatalyst (10 mg) was irradiated by fluorescent lamps in the presence of the cell suspensions (10 cm^3). Upon irradiation for 60 min in the presence of the SbTPP/p-SiO_2, the concentration of *Legionella* species apparently decreased from the initial concentration (6.4×10^5 CFU/100 cm^3) to 4×10^3 CFU/100 cm^3: its survival ratio was 0.6%. In the control experiments under irradiation in the absence of SbTPP/p-SiO_2, under dark conditions in the presence of SbTPP/p-SiO_2, and under dark conditions in the absence of SbTPP/p-SiO_2, nearly the initial concentration of *Legionella* species was kept. Thus, it is confirmed that SbTPP/p-SiO_2 has the photocatalytic activity to reduce concentrations of *Legionella* species.

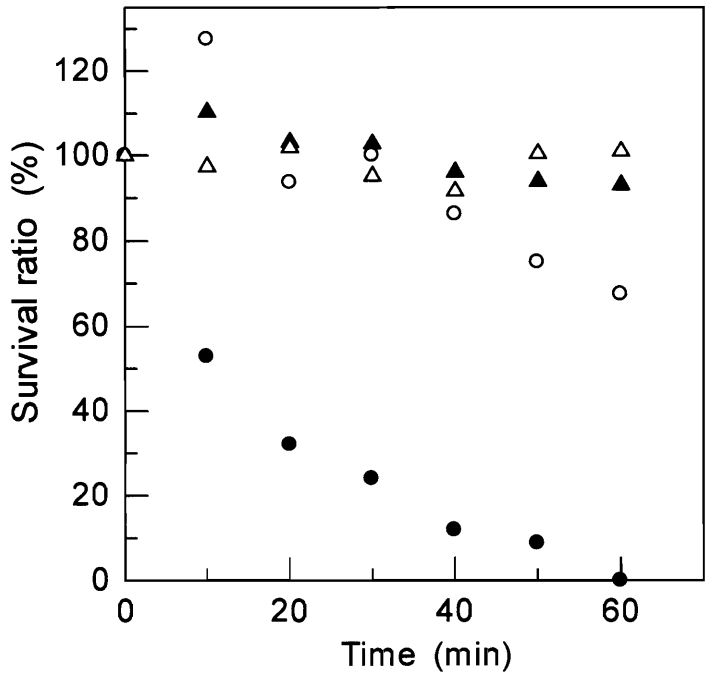

Figure 2-4. Bactericidal effect of SbTPP/p-SiO_2 catalyst on *L. pneumophila* under irradiation by fluorescent lamp in the presence of the catalyst (●), under the irradiation in the absence of the catalyst (○), under dark reaction in the presence of the catalyst (▲), and under dark reaction in the absence of the catalyst (△).

Practical experiments in the cooling-tower

The bactericidal effect of SbTPP/b-SiO_2 was substantiated in the practical experiments at a cooling-tower using a cylindrical photocatalytic bactericidal apparatus (Figure 2-3A). Under the conditions without any bactericidal treatments, *Legionella* species were found in the range of 20 to 139 CFU/100 cm^3 in the holder of the cooling tower, as shown in Figure 2-5. After the bactericidal apparatus was operated for 4 days, the concentration of *Legionella* species was reduced to 22 CFU/100 cm^3. Further operation reduced the concentrations of *Legionella* species to reach less than the

detection limit, and these levels were kept until the irradiation was stopped. Seven days after the irradiation was stopped, detectable amounts of *Legionella* species appeared. Thus, bactericidal effects of SbTPP/b-SiO_2 were practically confirmed in practical experiments involving cooling tower.

Practical experiments in the fountain

The preliminary concentrations of *Legionella* species naturally occurring in a public fountain were measured for 7 months from November, 2002 to May, 2003 under the conditions without any bactericidal treatments. Figure 2-6A showed the time-course plots of the concentrations of *Legionella* species, which were strongly dependent on atmospheric temperature. As the atmospheric temperature increased, the concentrations of *Legionella* species also increased, as shown in Figure 2-7. Concentrations of *Legionella* species exceeded 500 CFU/100 cm^3 in some samples when the atmospheric temperature went over 25 °C.

Practical bactericidal experiments were performed using a leaf-type photo-bactericidal apparatus containing 80 g of SbTPP/b-SiO_2, which was set into the fountain under sunlight irradiation during the period from May 28, 2003 to August 25, 2003. The concentration of *Legionella* species were reduced to less than the detection limit, 12

days after the leaf-type apparatus was installed to the fountain.

The concentrations of *Legionella* species were continuously kept at less than 30 CFU/100 cm^3 until the leaf-type apparatus was removed from the fountain on August 25 (Figure 2-6B). After the removal of the leaf-type apparatus, the concentrations of *Legionella* species were gradually increased to reach 100 CFU/100 cm^3, the environmental quality standard, 42 days after the removal of the leaf-type apparatus, as shown in Figure. 2-6B. In these practical experiments, it is noteworthy that 80 g of SbTPP/b-SiO_2 catalyst, *i.e.* 40 mg of $[SbTPP(OH)_2]^+Br^-$ chromophore, kept the concentration of *Legionella* species in 13 m^3 of water below 100 CFU/100 cm^3 for 120 days

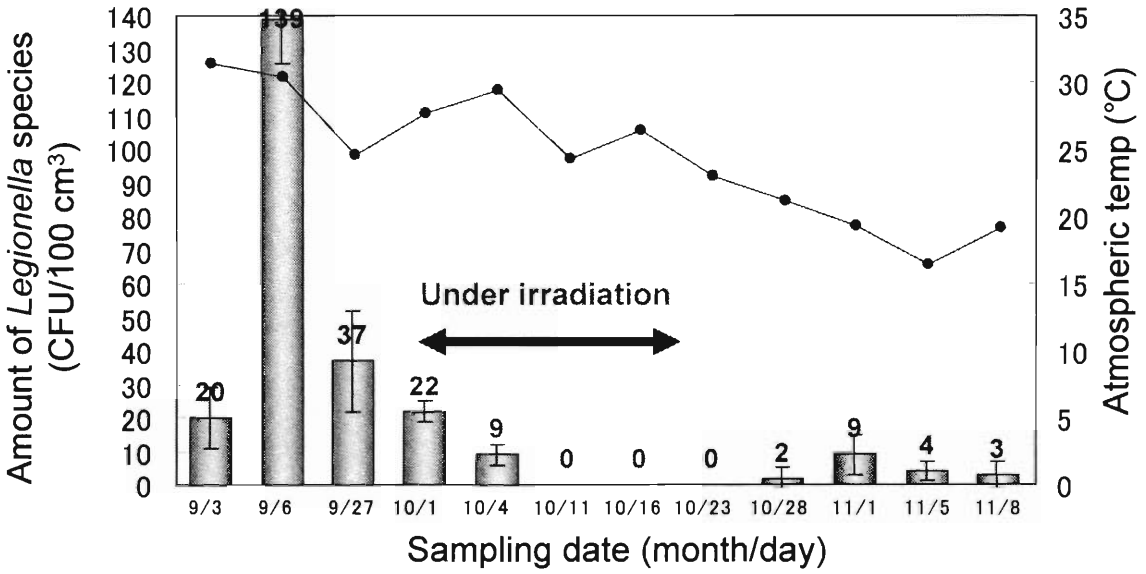

Figure 2-5. Time-course plots of the amounts of *Legionella* species in the cooling-tower along with the atmospheric temperature (●). The cylindrical photo-bactericidal apparatus was operated during October 1 to October 21, 2002. Conditions; catalyst: 4 kg, contents of water: ca. 800 dm^3, flow rate: 28 $dm^3 \cdot min^{-1}$, and the average retention time: 26 sec.

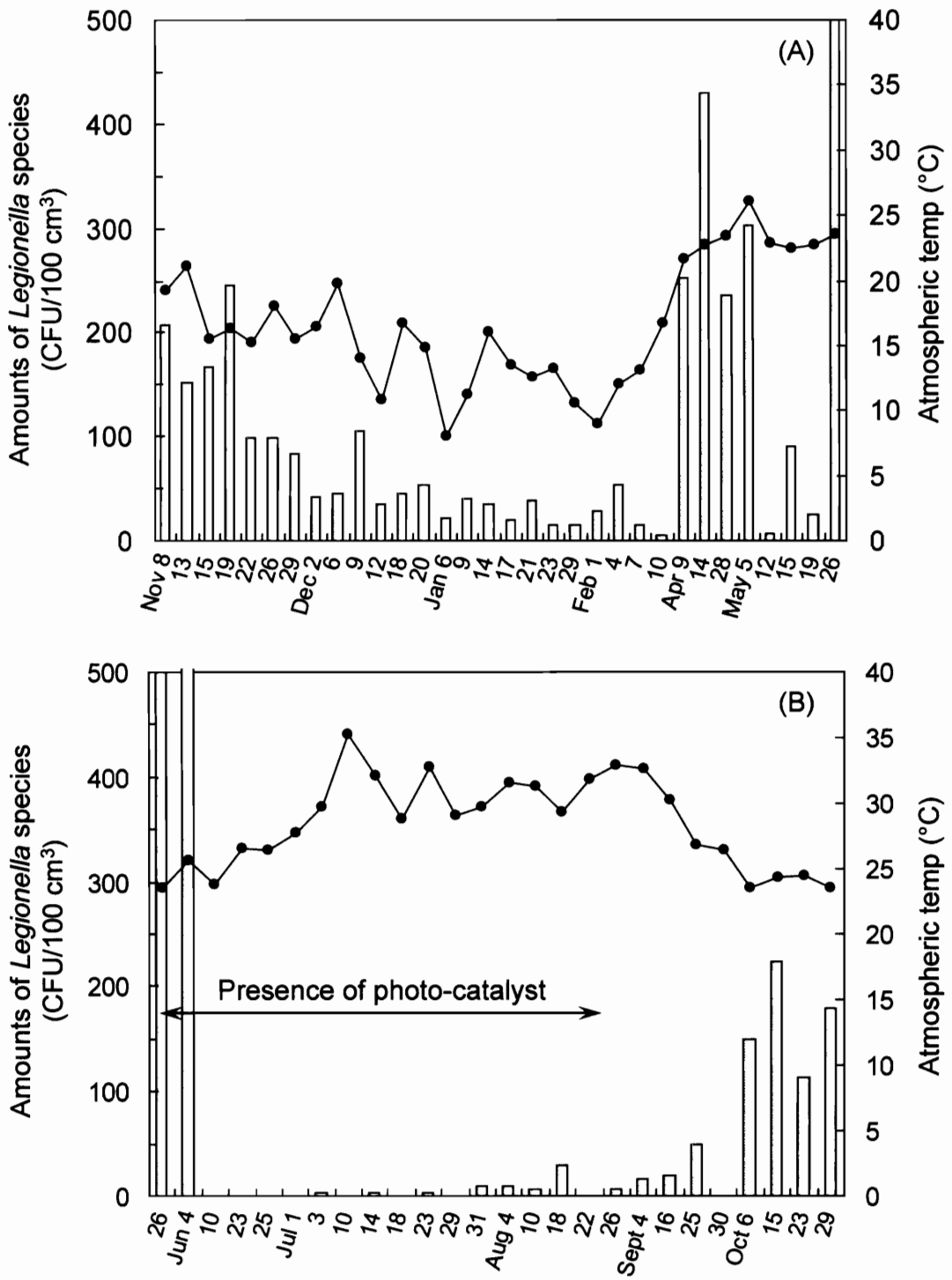

Figure 2-6. (A) Survey of *Legionella* species found in the fountain from November 26, 2002 to May 26, 2003. (B) The bactericidal experiment of *Legionella* species was performed using a leaf-type photo-bactericidal apparatus containing $SbTPP/SiO_2$ (80 g) under sunlight irradiation from May 26 to August 22, 2003.

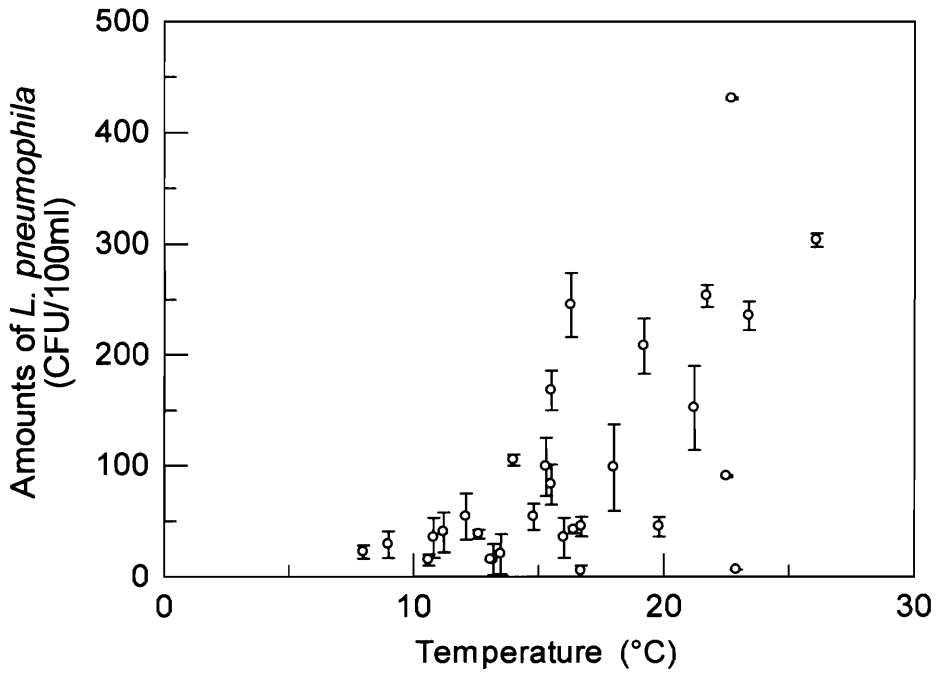

Figure 2-7. Dependence of amounts of *Legionella* species naturally occurring in the fountain on the atmospheric temperature. Bar (| — |) showed the standard deviation (SD): SD= $10\{[(q_1 - q_{av})^2 + (q_2 - q_{av})^2 + (q_3 - q_{av})^2] / 2\}^{1/2}$ where q_1, q_2, and q_3 denoted to colony numbers, and q_{av} denoted to average of q_1, q_2, and q_3.

Analysis of the catalyst

Elemental analysis of the catalyst before and after its use was performed by atomic absorption and ICP (Table 2-1). Before being used, the Sb content in SbTPP/b-SiO_2 was measured to be 80 ppm, which showed good agreement with the Sb content (72 ppm) calculated for the 0.05 wt% of SbTPP content in the catalyst. Contents of metals other than Sb in the catalyst were as follows: 67 (Na), 6 (Mg), 29 (Al), 12 (Ca), and 0.2 ppm (Fe). After being used in the practical experiment for 3

months in the fountain, the Sb content deceased from 80 ppm to 17 ppm. On the other hand, Na, Mg, Al, and Ca largely increased in the fountain where the following minerals were involved: Al = 2, Na =3, Ca = 18, Fe = 0.5, Mg = 9, Sb < 0.04 ppm, resulting in the occurrence of the ion-absorption on SiO_2. Moreover, the analysis of the SbTPP/b-SiO_2 catalyst used for the practical experiment in the fountain was performed by CLSM (Figure 2-8). It was found that the fluorescence come from the surface of the catalyst kept a shape similar to that of the original catalyst, but the intensity was weaker compared with the original spectra of SbTPP/b-SiO_2. On the other hand, the fluorescence from the inside of the catalyst maintained its original intensity. Therefore, it is suggested that $[SbTPP(OH)_2]^+$ chromophore was eliminated from the surface of catalyst. Irradiation of fluorescent light on the SbTPP/b-SiO_2 catalyst in deionized water did not entirely cause the spectral change and decrease of Sb content. Therefore, it is strongly suggested that the cationic $[SbTPP(OH)_2]^+$ chromophore was exchanged with alkali metal ions in the bulk water on the surface of catalyst under irradiation.

Table 2-1. Elemental analysis of SbTPP/SiO_2.[a)]

	Element [b)]					
	Na	Mg	Al	Ca	Fe	Sb
Before Use	67	6	29	12	0.2	80[c)]
After Use [d)]	208	79	89	316	0.5	13

a) SbTPP/SiO_2 photo-catalyst of which the SbTPP content was 0.05 wt%.
b) Content of elements in ppm.
c) This value showed good agreement to the Sb content (72 ppm) of 0.05 wt% of SbTPP.
d) The SbTPP/*b*-SiO_2 catalyst after the practical experiment in the fountain for 3 months.

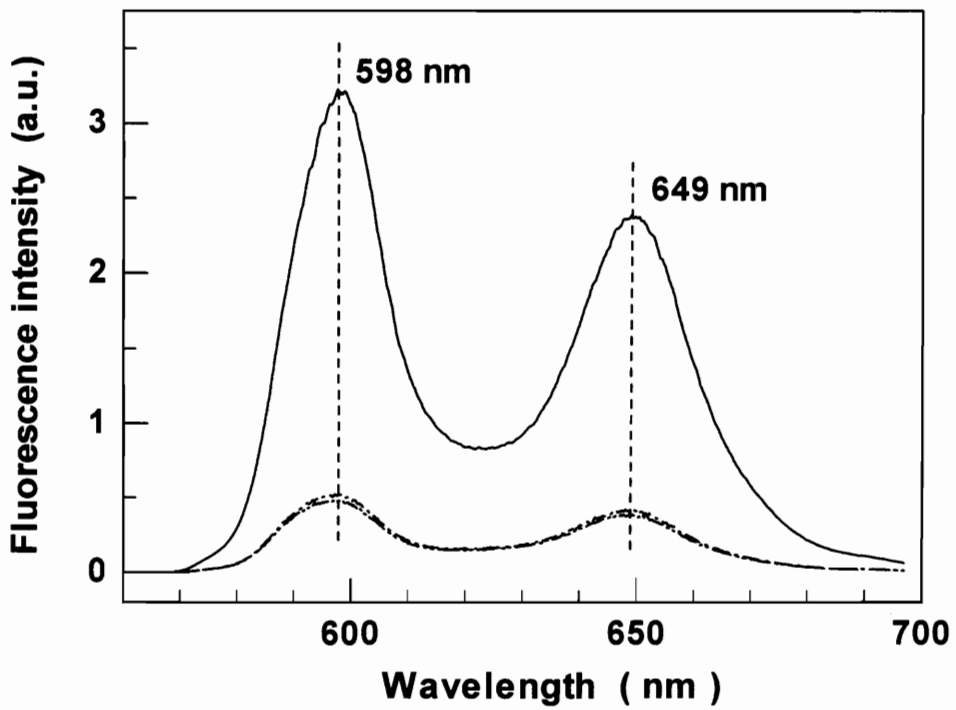

Figure 2-8. CLSM fluorescence spectra of SbTPP/*b*-SiO_2 before (solid line) and after use (dashed line) in the practical experiment in the fountain.

2-4. Conclusions

The SbTPP/*b*-SiO_2 catalyst operated effectively to reduce concentrations of *Legionella* spicies under visible-light irradiation in the living environments. It is well known that the photocatalytic process by TiO_2 has been widely applied to the disinfection and sterilization[9-12] as well as treatment of wastewater.[13] However, TiO_2 has only weak absorption in the visible region. It is, therefore, expected that the present visible-light bactericidal technique by the SbTPP/b-SiO_2 catalyst will be one of the powerful methods to sterilize aquatic bodies of harmful microbes.

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Chapter 3.

Bactericidal effect of silica gel-supported porphyrinatophosphorus(V) catalysts on *Escherichia coli* under visible light irradiation

3-1. Introduction

In previous chapters, it has been elucidated that SbTPP/SiO_2 catalyst has bactericidal effects on *E. coli* and *Legionella* species. However, antimony is a heavy metal and can potentially cause cancer. Sb_2O_3 is specified as being a carcinogenic substrate by the international agency research on cancer (IARC).[1] If antimony is diffused into a living environmental field, we have to take into account the accumulation of antimony in plants and animals. Therefore the author changes the primary metal of the catalyst from antimony to phosphorus to investigate bactericidal activity of tetraphenylporphyrinato phosphorus(V) immobilized on silica gel, which has a strong oxidizing ability.[2,3]

3-2. Experimental section

Instruments

^{1}H NMR spectra were taken on a Bruker AC 250P spectrometer. SIMS spectra were obtained on a Hitachi M2000A spectrometer. Microscopic spectroscopy was performed on an Olympus FV-300 confocal laser scanning microscope (CLSM) equipped with a spectrophotometer (STFL 250, Seki Technotron) linked to CLSM with an optical fiber.

Materials

Preparation of dialkyloxo(tetraphenylporphyrinato)phosphorus(V) chloride

According to previous literature,[4,5] dichloro(tetraphenylporphyrinato)-phosphorus(V) chloride, [PCl_2TPP]Cl, was prepared by the reaction of tetraphenylporphyrin (250 mg; H_2TPP) and $POCl_3$ (5 cm^3) in pyridine (50 cm^3) at reflux until the Soret band shifted from 416 to 438 nm in UV-Vis spectra. After cooling, the reactant was pored into hexane (700 cm^3). The resulting precipitate was collected and dissolved in $CHCl_3$ and washed with water. Crude [PCl_2TPP]Cl was obtained from the $CHCl_3$ solution after evaporation.

An MeCN-H_2O (3:1 v/v, 160 cm^3) solution of [PCl_2TPP]Cl (250 mg) was

heated at reflux until the Soret band shifted from 436 nm to 423 nm in UV-Vis spectra. After evaporation of MeCN, the aqueous solution was extracted with $CHCl_3$. The $CHCl_3$ extracts were combined and the $CHCl_3$ was evaporated to give dihydroxo(tetraphenylporphyrinato)phosphorus(V) chloride, $[P(OH)_2TPP]Cl$ (87% yield). An MeOH (250 cm^3) solution of $[PCl_2TPP]Cl$ (150 mg) was heated at reflux until the Soret band shifted from 435 nm to 424 nm in UV-Vis spectra. $[P(OMe)_2TPP]Cl$ was obtained at a 97% yield after the follow up procedure in a similar to that of $[P(OH)_2TPP]Cl$.

$[PCl_2TPP]Cl$.[5] 92% yield. λ_{max}/nm (logε) 435 (4.54). ^1H NMR δ 7.77–7.82 (m, 12H), 7.97–8.01 (m, 8H), 9.14 (d, J_{P-H} = 4.52 Hz, 8H). SIMS m/z 735 (PCl_2TPP).

$[P(OH)_2TPP]Cl$.[6] 87% yield. λ_{max}/nm (logε) 423 (4.51). ^1H NMR δ 7.67–7.70 (m, 12H), 7.99–8.03 (m, 8H), 8.80 (d, J_{P-H} = 1.93 Hz, 8H). SIMS m/z 676 ($P(OH)_2TPP - 2H$).

$[P(OMe)_2TPP]Cl$.[5] 97 % yield. λ_{max}/nm (logε) 424 (4.16). ^1H NMR δ –1.87 (d, J_{P-H} = 25.7Hz, 6H), 7.74–7.81 (m, 12H), 7.92–7.95 (m, 8H), 9.07 (d, J_{P-H} = 2.7 Hz). SIMS m/z 706 ($P(OMe)_2TPP$).

Preparation of SiO_2-supported (tetraphenylporphyrinato)phosphorus catalyst

The $[P(OH)_2TPP]Cl$ chromophore was supported on an SiO_2 carrier. Two types silica gel were used: silica-gel powders (p-SiO_2; 300 mesh, 0.04 mmϕ, 30 g, BW300, Fuji Silysia Chemical Ltd., Japan) and silica-gel beads (b-SiO_2; 0.85–1.70 mmϕ, 30 g, CARIACT Q–10, Fuji Silysia Chemical Ltd., Japan) (Table 3-2).

Into a toluene solution (400 cm^3) of $[P(OH)_2TPP]Cl$ or $[P(OMe)_2TPP]Cl$ (132 mg), p-SiO_2 (30 g) was added and then the solution refluxed for 18 h. The treated silica gel was filtered, and then dried under reduced pressure to give catalysts **1a** and **1b**, respectively, in which the content of $[P(OR)_2TPP]^+$ chromophore was 0.42 wt%.

A toluene–MeOH solution (4:1 v/v, 200 cm^3) of $[P(OH)_2TPP]Cl$ or $[P(OMe)_2TPP]Cl$ (13.2 mg) was refluxed with b-SiO_2 (30 g), filtered, and then washed with acetone and water (100 cm^3) to give **2a** and **2b**, respectively, where the content of the $[P(OR)_2TPP]^+$ chromophore was 0.042 wt%.

The catalysts were identified by observation of characteristic fluorescence peaks using CLSM.[7,8]

Table 3-1. Characterization of the catalyst

Catalyst	Chromophore (wt%)	Support [a)]
1a	$[P(OH)_2TPP]Cl$ (0.42)	p-SiO_2
1b	$[P(OMe)_2TPP]Cl$ (0.42)	p-SiO_2
2a	$[P(OH)_2TPP]Cl$ (0.042)	b-SiO_2
2b	$[P(OMe)_2TPP]Cl$ (0.042)	b-SiO_2
3	$[Sb(OH)_2TPP]Br$ (0.87)	p-SiO_2

a) p-SiO_2: powder type silica gel, size: 0.032–0.045 mmϕ, surface area: 429 $m^2 \cdot g^{-1}$. b-SiO_2: beads type silica gel, size: 1.70–4.00 mmϕ, surface area: 306 $m^2 \cdot g^{-1}$.

Photocatalytic sterilization of E. coli in an L-type glass tube

The amount of *E. coli* were adjusted to ca 10^4 cells·cm^{-3} by counting with microscope. The bactericidal activities of **1** on *E. coli* were examined in an L-type glass tube in a similar to chapter 1. Catalyst **1** (10 mg), 1.0 cm^3 of the cell suspension of *E. coli*, and 9.0 cm^3 of phosphate buffer (100 mmol·dm^{-3}, pH 7.0) were added into an L-type glass tube (length 18 cm, diameter 1.5 cm). The L-type glass tube was set on a reciprocal shaker and irradiated with two fluorescent lamps set above the shaker. The reaction temperature was kept constant at 30 °C. A portion (0.1 cm^3) of the solution was plated on the basal medium supplemented with agar (20 g·dm^{-3}), after which the colonies that appeared after incubation for 14 h at 30 °C were countered for three replicate plates.

Stability of catalysts ***2***

The catalysts (**2**; 15.1 mg) were immersed in aqueous NaCl and $CaCl_2$ (0.1 $mol \cdot dm^{-3}$; 20 cm^3) solutions and distilled water (20 cm^3) at room temperature. For each sampling, 10 cm^3 of solution was taken and subjected to the UV–Vis spectroscopy. After UV measurement, additional amounts of aqueous NaCl and $CaCl_2$ (0.1 $mol \cdot dm^{-3}$; 10 cm^3) solutions and distilled water (10 cm^3) were added to the original solutions to keep the amounts at 20 cm^3 and to maintain the stability experiment.

3-3. Results and discussion

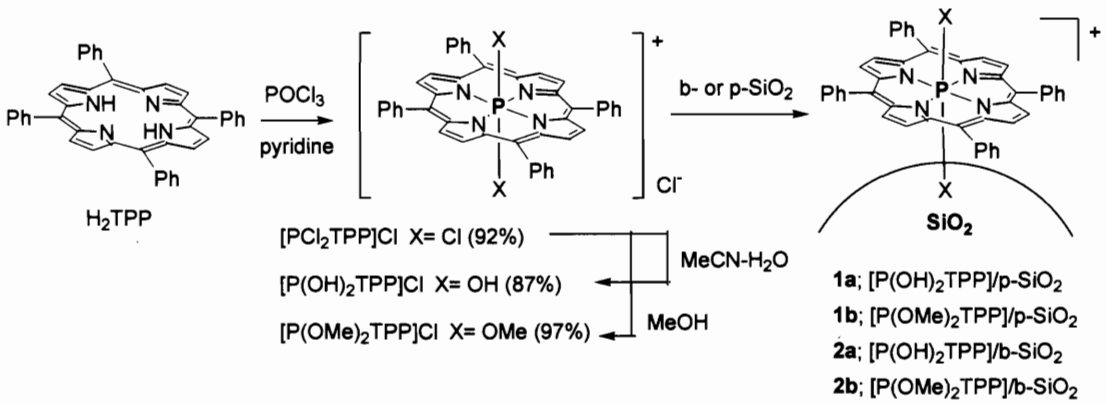
Preparation of the catalyst

According to the literature,[4,5] dichloro(tetraphenylporphyrinato)-phosphorus(V) chloride complex ([PCl_2TPP]Cl) was prepared by the reaction of tetraphenylporphyrin (H_2TPP) with $POCl_3$ in pyridine. The resulting [PCl_2TPP]Cl was converted into dihydroxo(tetraphenylporphyrinato)phosphorus(V) chloride ([$P(OH)_2$TPP]Cl) and dimethoxo(tetraphenylporphyrinato)phosphorus(V) chloride ([$P(OMe)_2$TPP]Cl) by solvolysis with H_2O and MeOH, respectively.

Immobilization onto silica-gel powder (p-SiO_2; 0.032–0.045 mmϕ) was performed by refluxing [$P(OR)_2$TPP]Cl in toluene with p-SiO_2 for 18 h. The treated silica-gel was filtered, and then dried under reduced pressure to give the p-SiO_2-supported $[P(OR)_2TPP]^+$ catalyst ([$P(OR)_2$TPP]/p-SiO_2; **1a** for R = H, **1b** for R = Me) in which the amount of the $[P(OR)_2TPP]^+$ chromophore was 0.42 wt% (Scheme 3-2).

$[P(OR)_2TPP]^+Cl$ (R=H and Me) was fixed on silica-gel beads (b-SiO_2, 0.85–1.7 mmϕ) that were larger in particle size than p-SiO_2 by refluxing in toluene-MeOH solution (4:1 v/v). After filtration, the catalysts were washed with acetone and water to give the b-SiO_2-supported $[P(OH)_2TPP]^+$ catalyst

([$P(OH)_2TPP$]/b-SiO_2; **2a**) in which the content of the $[P(OH)_2TPP]^+$ chromophore was 0.042 wt%. In a similar manner, $[P(OMe)_2TPP]Cl$ was fixed on b-SiO_2 to give the b-SiO_2-supported $[P(OMe)_2TPP]^+$ catalyst (**2b**). The catalysts were subjected to the stabilization experiments and the surface analysis.



Scheme 3-1. Preparation of the photocatalysts

Photocatalytic sterilization

The bactericidal effect of **1a** was investigated using *E. coli* K-12 (IFO3301). An aqueous phosphate buffer solution (10 cm^3) containing *E. coli* (ca. 10^4 cells·cm^{-3}) and **1a** (10 mg) was introduced into L-type glass tubes and irradiated by fluorescent lamp similar to the experiment involving the antimony analog ([$Sb(OH)_2TPP$]/p-SiO_2; **3**) described in chapter 1. Upon irradiation in the presence of **1a**, the amount of *E. coli* decreased with an increase in irradiation time, as shown

in Figure 3-1. In contrast, in control experiments in the presence of **1a** under dark conditions and in the absence of a catalyst under irradiation, the amount of *E. coli* remained at 100% of the survival ratio. Thus, photocatalytic bactericidal activity of **1a** was confirmed. In addition, bactericidal experiments were performed at various concentrations of the catalyst ([C]) under various light intensities (*a*). The results are summarized in Table 3-2.

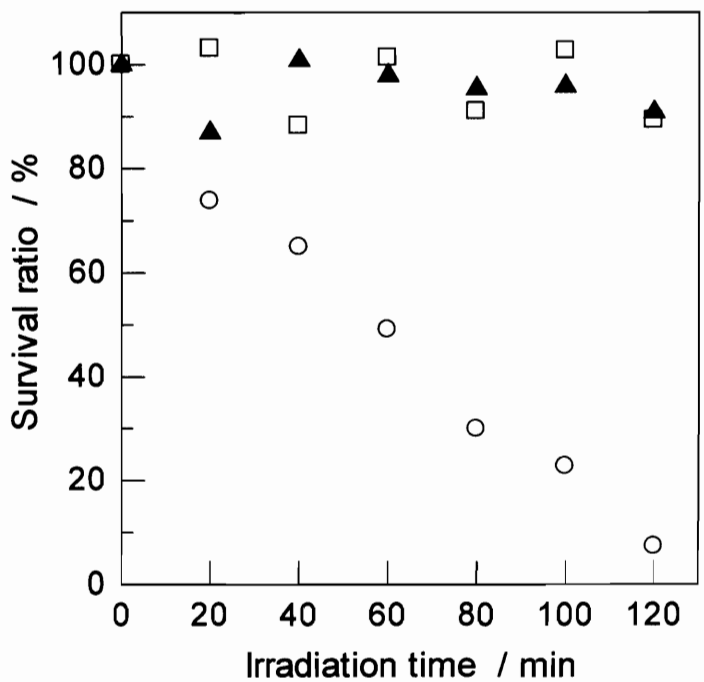


Figure 3-1. Bactericidal experiment for *E. coli* in the presence of **1a** under irradiation by a fluorescent lamp (○), in the absence of a catalyst under irradiation (□), and in the presence of **1a** under dark conditions (▲): Initial concentration of *E. coli* = ca. 1.0×10^4 cells·cm^{-3}, [**1a**] = 1.0 g·dm^{-3}, light intensity = 21 W·cm^{-2}.

Table 3-2. Photocatalytic sterilization of **1a**

a [a]	[C] [b]	a [C]	Amount of cell (B) (10^3 cells·cm^{-3})							slope
		Time (min) =	0	20	40	60	80	100	120	
21.0	0	0	7.77	7.64	7.60	7.58	7.67	7.58	7.54	1.3
21.0	0.1	2.1	8.27	8.11	7.60	7.38	7.21	7.06	6.79	12.8
21.0	0.5	10.5	7.75	7.02	6.57	6.06	5.75	5.29	4.93	25.4
21.0	1.0	21.0	7.60	6.36	5.38	3.95	2.60	1.32	0.50	60.9
21.0	2.0	42.0	7.76	6.05	4.41	1.42	0.22	0.02	0	96.0
21.0	3.0	63.0	8.57	6.37	3.34	0.63	0.06	0.01	0	126.0
7.3	1.0	7.3	4.97	4.64	4.34	4.01	3.86	3.54	3.31	14.3
11.0	1.0	11.0	5.14	4.90	4.39	3.97	3.73	3.25	2.66	19.4
17.0	1.0	17.0	5.20	4.75	4.04	3.50	3.07	1.98	1.25	30.9
20.3	1.0	20.3	5.56	4.66	3.74	2.31	1.83	1.24	0.24	43.8
25.6	1.0	25.6	5.79	4.50	3.55	2.40	0.92	0.30	0.05	59.0
21.0	2.0[c]	42.0	5.42	5.44	4.83	3.96	2.62	1.20	0.22	46.0
21.0	1.0[d]	21.0	10.31	7.25	5.81	4.44	2.47	1.21	0.66	92.5
21.0	5×10^{-7} [e]		5.28	5.11	4.25	3.83	2.62	1.64	0.86	39.0
21.0	1×10^{-6} [e]		5.92	4.50	4.32	3.27	1.88	0.87	0.48	50.0

a) Light intensity (a) in W·cm^{-2}.

b) Concentration of catalyst [C] in g·dm^{-3}.

c) Using **1b**. The k_2 value was determined to be 1.10 min^{-1}.

d) Using **3**. The k_2 value was determined to be 4.40 min^{-1}.

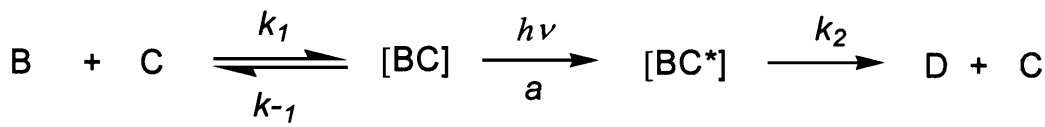
e) Using aqueous [P(OH)$_2$TPP]Cl solution (mol·dm^{-1}).

Kinetic analysis

As a working hypothesis, the author postulated a Michaelis-Menten-type mechanism, which involves the interaction between bacteria and the catalyst in the ground state was occurring (Scheme 3-2). Based on Scheme 3-2, the bactericidal reaction rate (v) was represented by eq. (1),

$$v = k_2 a[\mathrm{B}][\mathrm{C}] / (K_m + [\mathrm{B}]) \qquad (1)$$

where K_m and a values represent $(k_{-1} + k_2) / k_1$ and light intensity, respectively. The plots of the survival ratio of bacteria against the irradiation time at a given amount of **1a** showed a good straight line (Figure 3-1). The reaction rates are independent of the concentration of bacteria [B], and therefore, the bactericidal reaction obeys zero-order kinetics.



Scheme 3-2. Possible mechanism. B: living cells, C: catalyst, D: deactivated cells, a: light intensity.

If the absorption process of bacteria on the catalyst is very fast, e.g. k_1 is much larger than k_{-1} and k_2, the reaction rate (v) equals $ak_2[\mathrm{C}]$ (eq. (2)). Since the

$[P(OH)_2TPP]^+$ chromophore is cationic and SiO_2 has an affinity for bacteria, a strong interaction between the catalyst and the bacteria is expected. Therefore, eq. (2) can be transformed into eq. (3) where $[B_0]$ represents the initial concentration of *E. coli*. Actually, the plots with lower amounts of $[B_0]$–[B] against the irradiation time (t) gave a linear correlation until the conversion reached to 75%, as shown in Figure 3-2. The apparent rate constant (k_2) can be derived from the slope of the plot which equals $ak_2[C]$ (eq. (4)). As shown in Figure 3-3, the plots of the slope vs. $a[C]$ gave a straight line with a slope equal to k_2 which was determined to be 2.14 min^{-1}. In a similar way, the k_2 value was determined to be 1.10 and 4.40 min^{-1} for **1b** and **3,** respectively. The k_2 values of catalysts are listed in Table 3-3. Therefore, the catalytic reactivity increased in order of **1b** < **1a** < **3,** respectively.

$$v = -d[B]/dt = k_2a[C] \qquad (2)$$

$$[B_0] - [B] = k_2a[C]t \qquad (3)$$

$$\text{slope} = k_2a[C] \qquad (4)$$

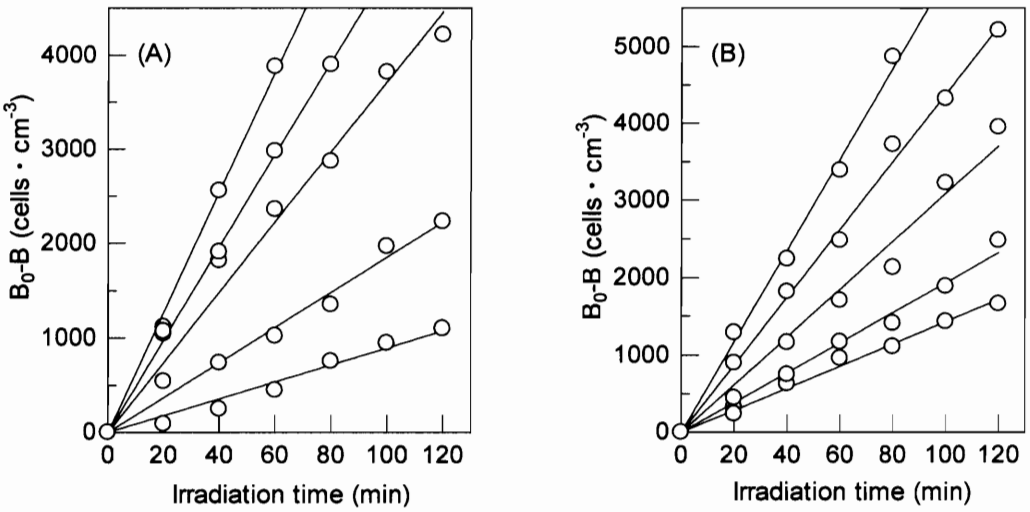


Figure 3-2. Plots of $[B_0]$–[B] against the irradiation time (t) (A) in the presence of a given amount of **1a** (0.1, 0.5, 1.0, 2.0, and 3.0 g·dm^{-3}) under irradiation with a light intensity of 21 W·cm^{-2} and (B) in the presence of 1 g·dm^{-3} of **1a** under various light intensities (a = 7.3, 11.0, 17.0, 20.3, and 25.6 W·cm^{-2}).

Table 3-3. Comparison of the k_2 values of catalysts

Catalyst	k_2 (min^{-1})
1a	2.14
1b	1.10
3	4.40

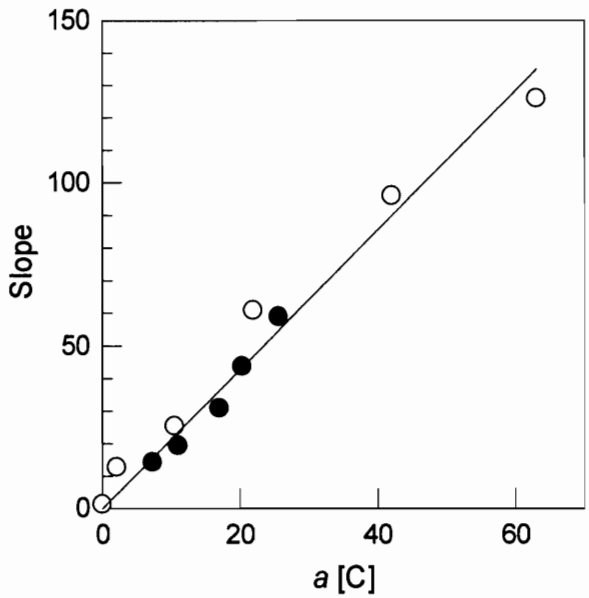


Figure 3-3. Plots of the slopes vs. the value of a[C] in the bactericidal experiments, using **1a** at the constant concentrations of catalyst ([C]; ●) or the constant light intensity (a; ○). The k_2 is 2.14 min^{-1}.

As mentioned above, the adsorption of *E. coli* on the catalyst is a key process. The $[P(OH)_2TPP]^+$ itself may have a strong affinity to the bacteria, so the author studied the sterilization of *E. coli* with $[P(OH)_2TPP]Cl$ in an aqueous buffer solution. The plots of $[B_0]$–[B] *vs.* irradiation time produced a straight line similar to that of the **1a** (Figure 3-4). Therefore, the cationic $[P(OH)_2TPP]^+$ chromophore itself strongly interacts with the bacteria which is why the effective sterilization occurred.

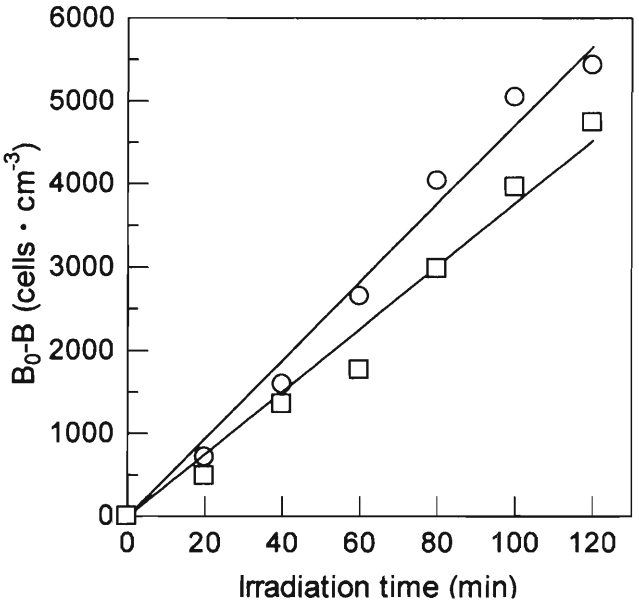


Figure 3-4. Plots of B_0–B vs. irradiation time using $[P(OH)_2TPP]Cl$ whose concentrations were 1×10^{-6} (○) and 5×10^{-7} mol·dm^{-3} (□): initial concentration of *E. coli* = 5.6×10^3 cells·cm^{-3}; light intensity = 21 W·cm^{-2}.

Mechanism

Under N_2, photochemical sterilization using **1b** did not occur (Figure 3-5B). Therefore, activated oxygen appears to participate in the sterilization process involving **1b**. Hirakawa *et al.* have reported the photochemical damage of DNA occurred with $[Sb(OH)_2TPP]^+$ due to singlet oxygen ($^1O_2$) which was generated by energy transfer from the excited triplet state of $[Sb(OH)_2TPP]^+$ to O_2.[9] In the present case, therefore, the bactericidal effect of **1b** was mainly attributed to the activated oxygen involving $^1O_2$. In contrast, **1a** exhibited considerable bacterial effect in the absence of O_2, as shown in Figure 3-5A. Another reaction pathway through the interaction of the axial hydroxo ligand with bacteria may also be

involved in the case of **1a**.

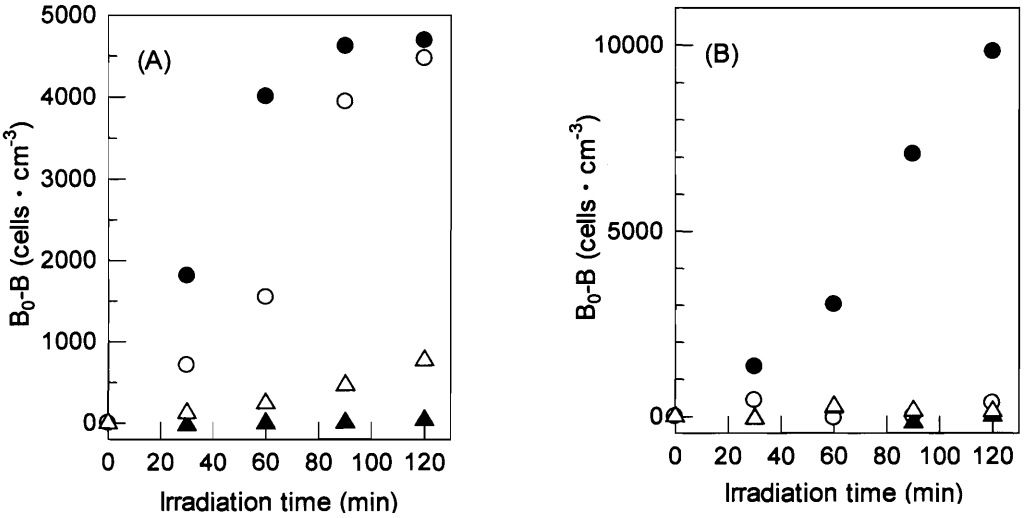
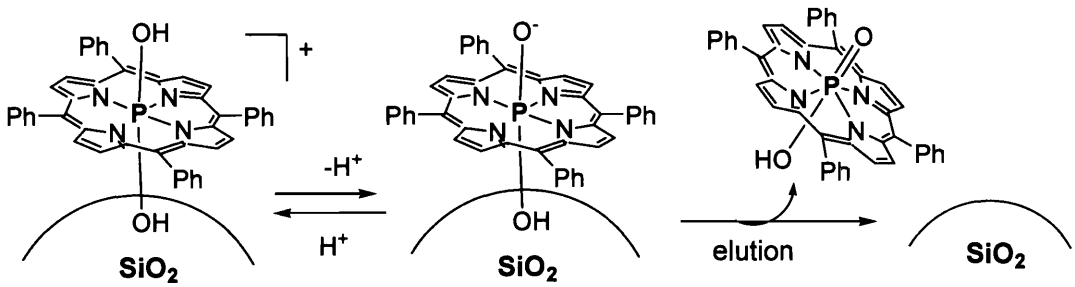


Figure 3-5. Photochemical sterilization of *E. coli* by (A) **1a** and (B) **1b** in the presence of visible light under an aerobic atmosphere (●) and under a nitrogen atmosphere (○) and in the dark condition under an aerobic atmosphere (▲) and a under nitrogen atmosphere (△).

Stability of the catalyst

A catalyst must be stable in order to use in practical applications. The catalysts (**2**; 15.1 mg) were stored in aqueous solutions of NaCl and $CaCl_2$ (0.1 mol·dm^{-3}; 20 cm^3) and distilled water (20 cm^3) at room temperature. Time-course plots were shown in Figure 3-6. The amount of $[P(OH)_2TPP]^+$ chromophore eluted from the catalyst was measured by its absorbance in UV-Vis spectra of the aqueous solutions. The eliminated porphyrin chromophore was determined to be $[P(OH)_2TPP]^+$ catalyst and not to be the free base tetraphenylporphyrin (H_2TPP).

In the case of **2a**, the amounts of $[P(OH)_2TPP]^+$ chromophore in the aqueous solution were gradually increased as the elution time increased. In particular, >80% of $[P(OH)_2TPP]^+$ chromophore were eliminated after one month in the aqueous NaCl and $CaCl_2$ solutions. However, the elution of the $[P(OMe)_2TPP]^+$ chromophore from **2b** was less than 20% after the elution for 35 days, as shown in Figure 3-6. Yasuda and co-workers have already elucidated that the deprotonation of an axial O–H ligand of $[P(OH)_2TPP]^+$ occurs, especially under irradiation.[6,10-11] Therefore, because the deprotonation causes the catalyst to become neutral, the elimination from **2a** was accelerated compared to that from **2b** (Scheme 3-3). The stability of MTPP (M= Sb, P) can be attributed on dissociation constant of hydroxyl ligand on metalloporphyrin. The OH group on PTPP is more acidic than that on SbTPP; The pKa value = 9.5 for PTPP, pKa = 10.3 for SbTPP.[12]



Scheme 3-3. Plausible mechanism for elution of $[P(OH)_2TPP]^+$ from the catalysts

Although total amounts of $[P(OMe)_2TPP]^+$ chromophore did not appreciably change in the case of **2b** in the elution experiments over a few weeks (Figure 3-7), confocal laser scanning microscope (CLSM) analysis showed that the fluorescence coming from the shallow part (100 μm inside the surface) of **2b** was getting weaker as elution time increased (Figure 3-7). In contrast, the fluorescence intensity from the deep part (400 μm inside) of the catalyst did not decrease appreisiably. Moreover, a dimple appeared on the silica-gel surface after the stability experiment in aqueous $CaCl_2$ and NaCl solutions, suggesting the elimination of SiO_2.

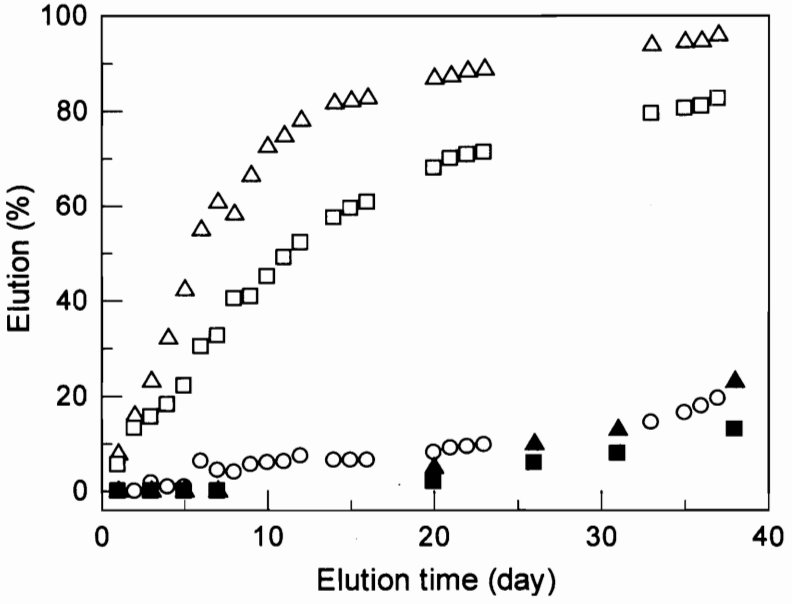


Figure 3-6. Time-course plots of the amount of $[P(OH)_2TPP]^+$ eluted from **2a** in the aqueous $CaCl_2$ solution (△), aqueous NaCl solution (□), and distilled water (○) and from **2b** in the aqueous $CaCl_2$ solution (▲) and aqueous NaCl solution (■). The amounts were determined by UV-Vis spectrometer using the absorption coefficiency of the $[P(OH)_2TPP]$ chromophore at 424 nm ($\varepsilon = 1.17 \times 10^5$ $dm^3 \cdot mol^{-1} \cdot cm^{-1}$).

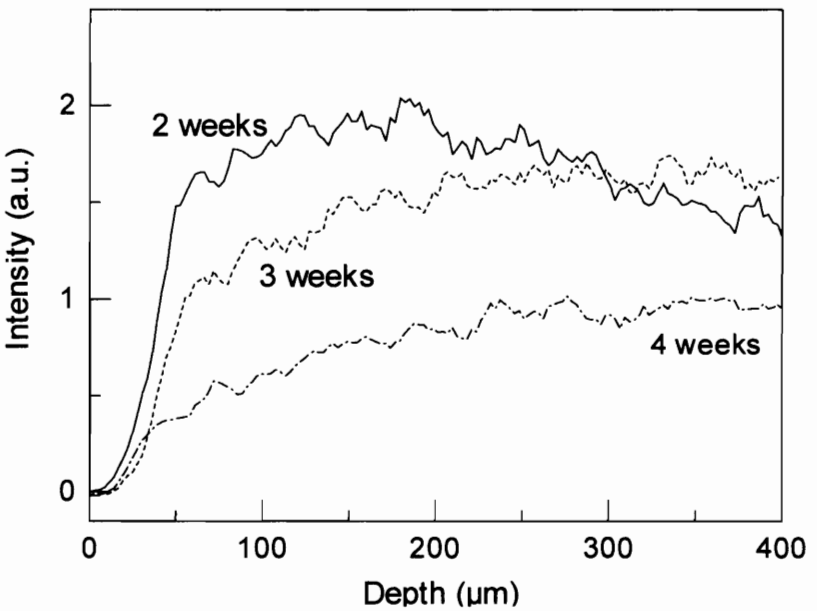


Figure 3-7. Distribution of fluorescence in depth from the surface of **2b** after the elution experiment for a given periods.

3-5. Conclusions

[P(OR)TPP]/SiO_2 catalysts with high stability and non-toxicity, i.e., 50% of lethal dose (LD_{50}) of [$P(OH)_2$TPP]Cl > 2000 $mg \cdot kg^{-1}$,[13] showed remarkable bactericidal activity towards *E. coli* under visible-light irradiation. Thus, it is expected that the present visible-light bactericidal technique will contribute to the practical sterilization of harmful microbes in aquatic bodies.

3-5. References and footnote

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13 Fifty percent of lethal dose (LD_{50}) of $[P(OH)_2TPP]Cl$ was examined by Japan food research laboratories for use in male and female mice on 2005 (404110082-001).

Summary

This thesis on "Photocatalytic bactericidal effect of silica gel-supported metalloporphyrin complexes" is summarized as follows:

Chapter 1 dealt with a silica gel-supported dihydroxo(tetraphenylporphyrinato)antimony(V) complex ($SbTPP/SiO_2$), which operated a bactericidal agent under visible light irradiation. The $SbTPP/SiO_2$ particles irradiated by fluorescent light in a test tube induced remarkable bactericidal activity for *E. coli* cells. The bactericidal activity of the $SbTPP/SiO_2$ was affected by both the concentration of the $SbTPP/SiO_2$ and the light intensity. Under irradiation by visible light, the $SbTPP/SiO_2$ photocatalyst showed much superior bactericidal activity to the commercially available TiO_2. Moreover, under irradiation by sunlight, bactericidal activity of the $SbTPP/SiO_2$ was observed, and the bactericidal effect of the $SbTPP/SiO_2$ particles was effective for continuous treatment on a column photoreactor under fluorescent-light irradiation.

Chapter 2 dealt with the bactericidal effect of $SbTPP/SiO_2$ on *Legionella* species. Experiment to reduce concentrations of *Legionella* species were performed using a cylindrical $SbTPP/SiO_2$-photocatalytic bactericidal apparatus in a cooling tower which held 800 dm^3 of water. After 10 days, the concentrations of *Legionella* species were reduced to less than the detection limit, and these levels were kept until the irradiation was stopped. Also, a photocatalytic bactericidal experiment was conducted with a fountain that was filled with 13 m^3 of water. The concentrations of *Legionella*

species were reduced to less than the detection limit 12 days after the $SbTPP/SiO_2$ catalyst was installed in the fountain receiving sunlight irradiation. The concentrations of *Legionella* species were kept at less than 30 CFU/100ml for 3 months until the catalyst was removed from the fountain. Thus, visible-light irradiation of the $SbTPP/SiO_2$ catalyst induced a remarkable bactericidal activity against *Legionella* species in the living environment.

Chapter 3 dealt with dihydroxo- and dimethoxo(tetraphenylporphyrinato)-phosphorus(V) complexes ($[P(OR)_2TPP]^+$) immobilized on silica gel ($[P(OR)_2TPP]/SiO_2$). In the case of $[P(OH)_2TPP]/SiO_2$, the amount of *E. coli* decreased linearly versus the irradiation time, showing that the bactericidal reaction obeyed zero-order kinetics. Adsorption of bacteria on the catalyst is thought to be a key pathway by analysis according to Michaelis-Menten's equation. $[P(OMe)_2TPP]/SiO_2$ was more effective for sterilization than $[P(OMe)_2TPP]/SiO_2$. Stabilities of the $[P(OR)_2TPP]^+$ immobilized on silica gel beads were investigated in aqueous $CaCl_2$ and NaCl solutions. The elution of the $[P(OH)_2TPP]^+$ chromophore from complex $[P(OH)_2TPP]/SiO_2$ was faster than it was from $[P(OMe)_2TPP]/SiO_2$. The catalyst $[P(OMe)_2TPP]/SiO_2$ with high stability and non-toxicity showed remarkable bactericidal activity towards *E. coli* under visible light irradiation.

In conclusion, the author has considered visible light driven bactericidal effect of silica-gel supported metalloporphyrin complexes ($MTPP/SiO_2$) for *E. coli* and *Legionella* species from fundamental and practical stand points of view. As the results, the author have elucidated that the present bactericidal technique has the following unique features.

1) Silica gel is most suitable for visible light-driven catalyst support because silica gel is transmitted visible light effectively and immobilize porphyrin chromophores. Also, SiO_2 is easy to control the surface area, the pore volume, and the pore diameter which the attributes of these porphyrin complexes are required.
2) SbTPP and PTPP had high affinity with microorganisms owing to their cationic complexes.
3) The sterilization was able to be performed under the visible light irradiation.
4) Antimony porphyrin complex and phosphorous porphyrin complex had visible light-driven bactericidal effect. The bactericidal effect was mainly attributed to singlet oxygen $^1O_2$ which was generated by energy transfer from the excited triplet state of MTPP (M= Sb, P) to O_2.
5) Since the toxicity of SbTPP and PTPP are low, we can use safety these catalysts in living environmental fields.

List of papers

The contents of this thesis are composed of the following papers.

1. Bactericidal effect of a silica gel-supported porphyrinatoantimony(V) complex under visible light irradiation

Haruhiko Yokoi, Tsutomu Shiragami, Jun Hirose, Takeshi Kawauchi, Kenichi Hinoue, Yoshiyuki Fueda, Kazunori Nobuhara, Izumi Akazaki, and Masahide Yasuda,

World J. Microbiol. Biotechnol., **19**, 559–563 (2003).

2. Visible-light bactericidal effect of silica gel-supported porphyrinatoantimony(V) catalyst on *Legionella* species occurring in the living environmental fields

Yoshiyuki Fueda, Manabu Hashimoto, Kazunori Nobuhara, Haruhiko Yokoi, Yasuhiro Komiya, Tsutomu Shiragami, Jin Matsumoto, Kimiko Kawano, Sen Suzuki, and Masahide Yasuda,

Biocontrol Sci., **10,** 55-60 (2005).

3. Bactericidal effect of silica gel-supported porphyrinatophosphorus(V) catalysts on *Escherichia Coli* under visible light irradiation

Yoshiyuki Fueda, Hideshige Suzuki, Yasuhiro Komiya, Yuka Asakura, Tsutomu Shiragami, Jin Matsumoto, Haruhiko Yokoi, and Masahide Yasuda,

Bull. Chem. Soc. Jpn., **79,** 1420-1425 (2006).

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