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RESEARCH NOTE

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Antibacterial Effect of TiO_2 Photocatalytic Reactor against Food-borne Pathogens

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Abstract Titanium dioxide (TiO_2) shows antibacterial effects when exposed to near ultra violet (UV) light. In this study, TiO_2 photocatalytic continuous reactor was designed and applied to food-borne pathogens such as *Vibrio parahaemolyticus* ATCC 17802, *Salmonella choleraesuis* ATCC 14028, and *Listeria monocytogenes* ATCC 15313. TiO_2 films were prepared by conventional sol-gel dip-coating method using titanium tetra iso-propoxide (TTIP). The antibacterial activity of photocatalytic reactor with various flow rates and UV-A illumination time showed effective bactericidal activity. As the UV-A illumination time increased, survival rates of those bacteria decreased. After 60 min of UV-A illumination, the survival rates of *V. parahaemolyticus* and *S. choleraesuis* were less than 0.1%. However, that of *L. monocytogenes* was about 5% at that time point. These results present an effective way to exclude pathogenic bacteria from aqueous foods.

Keywords: TiO_2 photocatalyst, bactericidal, *Salmonella choleraesuis*, *Vibrio parahaemolyticus*, *Listeria monocytogenes*

Introduction

Waste water from hospital, food factories, and other contaminated sites sometimes contains microorganisms, virus, and some putrefiable organic compounds. Many methods have been developed to eradicate harmful microorganisms from water and foods in various fields. Among these, one of the typical methods is the photo-sterilization by ultraviolet (UV), which provides a high rate of sterilization at room temperature. However, direct use of UV lights with short wave lengths also has negative effects on the human body. As viewed from crystalline structure, TiO_2 is a multi-crystal form substance. It includes brookite, rutile, and anatase, although only rutile and anatase are widely used. Both rutile and anatase forms have a square crystal system structure. The crystal density and absorption ability of UV-visible light of rutile are greater than those of anatase, but anatase possesses relatively high reflectivity for UV-visible light. Alternately, it is well known that the TiO_2 in anatase crystalline form is capable of oxidizing and decomposing organic compounds (1-3).

Vibrio parahaemolyticus and *Salmonella choleraesuis* are prevalent pathogens of porcine and marine foods. Environmental strains of *V. parahaemolyticus* are typically not human pathogens. However, these strains cause disease in shrimps, oysters, mussels, and other marine invertebrates and fishes (4). *S. choleraesuis* is an important component of the porcine respiratory disease complex (PRDC). The recognition of *S. choleraesuis* as an important and common cause of swine respiratory disease and the emergence of porcine reproduction and respiratory syndrome (PRRS) as a new swine disease have both occurred only relatively recently (5). *Listeria monocytogenes* is a Gram-positive facultative intracellular pathogen that is also the causative agent of food borne listeriosis (6). TiO_2 photocatalysts have attracted great attention as an alternative material to aid in the purification of water and air (7-11). TiO_2 photocatalysts generate strong oxidizing power when illuminated with UV light with wavelength of less than 385 nm (12). Illuminated TiO_2 photocatalysts decompose organic compounds by oxidation, with holes (h^+) generated in the valence band and with conduction hydroxyl radical ($OH^\bullet$) produced by the oxidation of water.

TiO_2 particles have been reported to catalyze the decomposition of some bacteria (13-15) and cancer cells (16,17) with the illumination of near-UV light, probably due to the generation of free radicals by photoexcited TiO_2 particles. Reports have appeared concerning the bactericidal effects of TiO_2 powder, often referring to $OH^\bullet$ as the toxic agent (18). In 1985, Matsunaga *et al.* (19) reported that microbial cells in water could be killed by contact with a TiO_2-Pt catalyst upon illumination with near-UV light for 60 to 120 min. Since then, various researches on antibiotic effects of TiO_2 photocatalyst have been intensively conducted on a wide spectrum of organisms including viruses, bacteria, fungi, algae, and cancer cells. Most of these studies used the conventional powder photocatalyst, commonly the Degussa P25. In the previous study, TiO_2 powers are used as the photocatalyst for sterilization in the batch photocatalytic reactor. These photocatalyst powders, however, are difficult to separate and recover after disinfection of microorganisms. Immobilization of TiO_2 on an inert substrate, therefore, to form a bactericidal surface seems to be a practical solution.

In this study, we have coated transparent TiO_2 thin film on the surfaces of alumina balls using sol-gel dip-coating

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method and designed a photocatalytic reactor in order to demonstrate the bactericidal effect with various flow rates of cell suspension and illumination times of near-UV light. Bactericidal activity on the food borne pathogens was evaluated by the inactivation of 3 strains of *V. parahaemolyticus*, *S. choleraesuis* subsp., and *L. monocytogenes*.

Materials and Methods

Preparation of TiO_2-coated alumina balls The photocatalytic property was provided to the alumina balls (Φ 8 mm, Asia Abrasive Co., Ltd., Korea) by depositing TiO_2 film on the surfaces of the balls. TiO_2 films were coated by conventional sol-gel dip-coating method. TiO_2 sol was prepared by using H_2O, HNO_3, and titanium tetra isopropoxide (TTIP). A solution with the ratio of 150 mL of H_2O: 15 mL of TTIP: 1 mL of HNO_3 was heated at 80°C for 24 hr avoiding vaporization. The precursor solutions were transparent and were very stable in air. Pre-cleaned alumina balls were immersed into the TiO_2 sol for 30 min and dried overnight in desiccator followed by baking at 600°C for 2 hr. This procedure was repeated 3 times. Anatase crystalline structure of TiO_2 film deposited on the surfaces of alumina balls was confirmed by X-ray diffraction analysis.

Microorganisms and culture conditions *V. parahaemolyticus* ATCC 17802, *S. choleraesuis* subsp. ATCC 14028, and *L. monocytogenes* ATCC 15313 were purchased from Korean Culture Collection of Microorganisms (KCCM, Seoul, Korea). The culture media for seed cultures of *V. parahaemolyticus*, *S. choleraesuis* subsp., and *L. monocytogenes* were tryptic soy broth (TSB, Merck, Darmstadt, Germany) with 3%(w/v) NaCl, TSB, and brain heart infusion (BHI, Becton Dickinson, Franklin Lakes, NJ, USA), respectively. The working culture was prepared by inoculating 3 mL of each overnight seed culture to 250-mL glass flasks containing 50 mL of media. Culture flasks were aerobically incubated on a rotary shaker at 37°C for 24 hr. Detail of the planktonic cell preparation was described in previous paper (20).

Bactericidal activity of photocatalytic reactor The photocatalytic reaction system consisted of sampling tube (A), varistaltic pump (B), thermo-circulator (C), UV power supply (D), photocatalytic reactor (E), cell suspension vessel (F), magnetic stirrer (G), and UV lamp (H) (Fig. 1A). Figure 1B shows a schematic diagram of triple annular photocatalytic reactor. The interior chamber consists of quartz and contains the light source. Quartz allows for optimal UV light permeation. Secondary chamber that contains TiO_2-coated alumina balls consists of glass and allows flow of the cell suspension. Outer chamber is also glass and made for the cooling material (water). The reactor volume of secondary chamber was approximately 700-mL. Bacterial cells suspended in 1,500 mL of phosphate buffered saline (PBS, 10^8 CFU/mL) were allowed to flow into the reactor at flow rates of 100, 250, and 500 mL/min. This reactor was illuminated with a UV lamp (F18T8BLB/580, 18 W, Sankyo Denki Co., Ltd., Kanagawa, Japan). The light intensity, which peaks at 365 nm, was 10 W/m^2 in the alumina ball located between light source and reactor cooling jacket. Temperature of the reactor was maintained at 37°C using the thermo-circulator. For the control groups, the reactions were performed in the dark or with alumina balls that do not have TiO_2-coating. One mL of bacterial cell suspension was taken at every 30 min for 3 hr. For viable cell counting serial dilution of the bacterial suspension was carried out with PBS. Fifty μL of each dilute was poured on each agar plate and incubated at 37°C for overnight. Colony forming unit (CFU) was counted. Survival rate was calculated by percentage of the initial CFU over the final CFU. All the reactions were performed 3 times.

Results and Discussion

Bactericidal activity of *V. parahaemolyticus* The bactericidal activity of the photocatalytic reactor on food-borne pathogenic bacteria was evaluated. The flow and recycling of the bacterial cell suspensions into the reactor continued for 3 hr. Cell viabilities of *V. parahaemolyticus* after passing through the reactor in the dark, UV-A with TiO_2-noncoated balls, and UV-A with TiO_2-coated balls at various flow rates were shown in Fig. 2. With the flow rate of 100 mL/min (Fig. 2A), more than 95% of *V. parahaemolyticus* lost their viability within 30 min in the case of TiO_2-coated balls with UV illumination. Cell viability was rapidly decreased until 30 min, and no detectable organism was observed after 60 min when compared with a control reaction performed in the dark. However, UV light itself showed slight bactericidal effect. Five % of the cells lost their viability at 30 min and the survival rate was decreased down to 76% at 60 min when compared with a control reaction performed in the dark. From these results it is obvious that feasible synergistic effect might be achieved with the use of UV-A light and TiO_2 photocatalytic films. For the flow rate of 250 mL/min

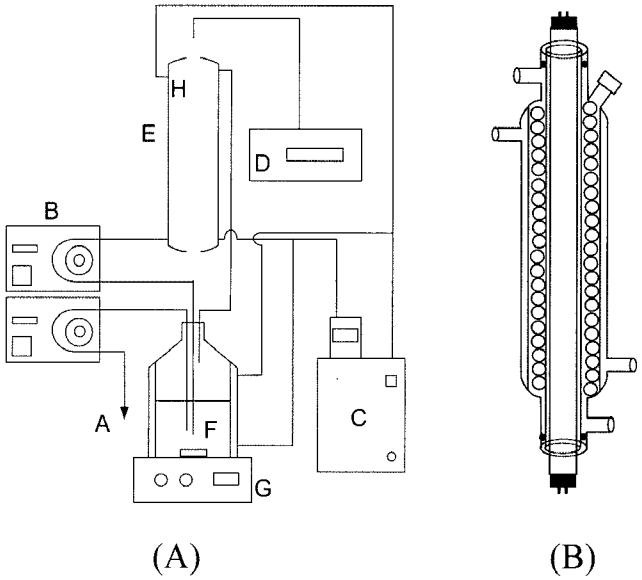

Fig. 1. Experiment apparatus for photocatalytic reaction system. (A) Schematic diagram of photocatalytic reaction system, (B) schematic diagram of triple annular photocatalytic reactor. A, Sampling tube; B, varistaltic pump; C, thermo-circulator; D, UV power supply; E, photocatalytic reactor; F, cell suspension vessel; G, magnetic stirrer; H, UV lamp.

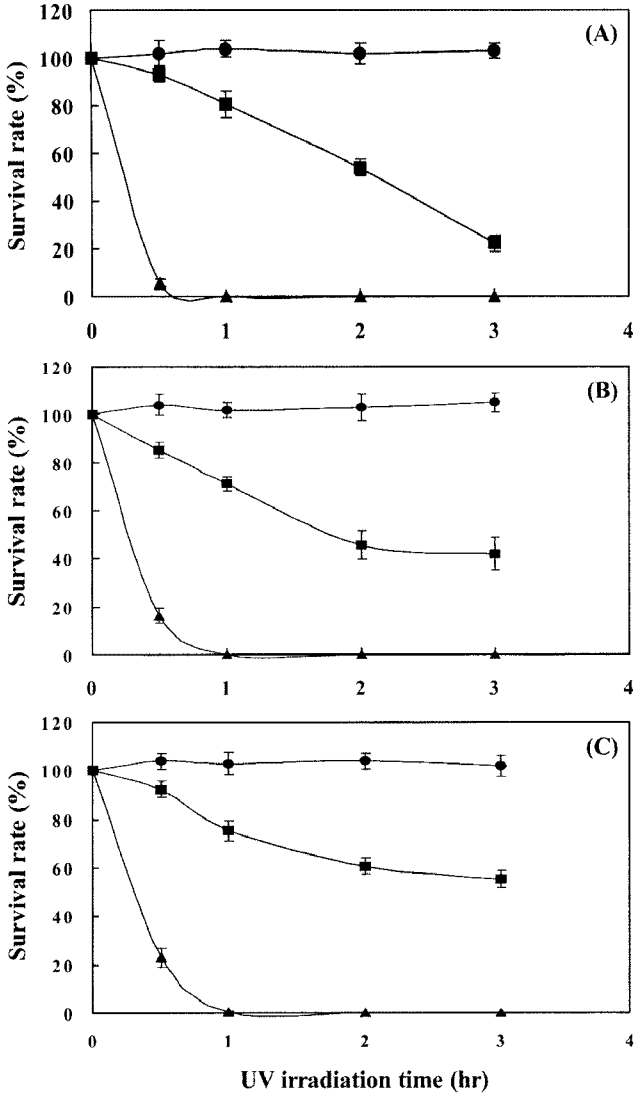

Fig. 2. Bactericidal effect of photocatalytic reactor depending on the UV-A illumination time and flow rate of *V. parahaemolyticus* suspension. Error bars represent standard deviations. (A), 100 mL/min; (B), 250 mL/min; (C), 500 mL/min. (●, dark; ■, UV; ▲, UV+TiO_2)

(Fig. 2B), survival rate was reached 18% at 30 min and for 500 mL/min, 22% of survival rate was observed (Fig. 2C). Negative linear correlation between flow rate and bactericidal activity of the photocatalytic reactor was observed. As the flow rate of cell suspension increased 5 times, survival rate of the organism also increased about 5 times. However, at all flow rates no viable cells were detected after 60 min exposure to UV-A and TiO_2 alumina balls. All subsequent experiments were done at the flow rate of 100 mL/min.

Bactericidal activity of *S. choleraesuis* subsp. The survival rates of *S. choleraesuis* subsp. are illustrated in Fig. 3. After 30 min of illumination with UV light, *S. choleraesuis* subsp. showed 10% of survival rate. *S. choleraesuis* subsp. showed lower sensitivity to the photocatalytic reactor than *V. parahaemolyticus*. Furthermore, about 3% of survival rate was observed at 60 min. No detectable organism was observed after 2 hr of reaction.

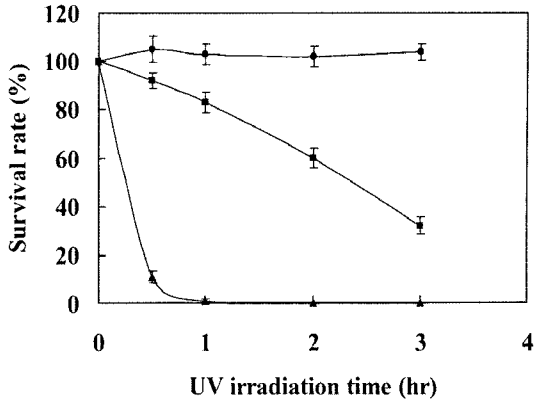

Fig. 3. Bactericidal effect of photocatalytic reactor on *S. choleraesuis* subsp. Error bars represent standard deviations. (●, dark; ■, UV; ▲, UV+TiO_2)

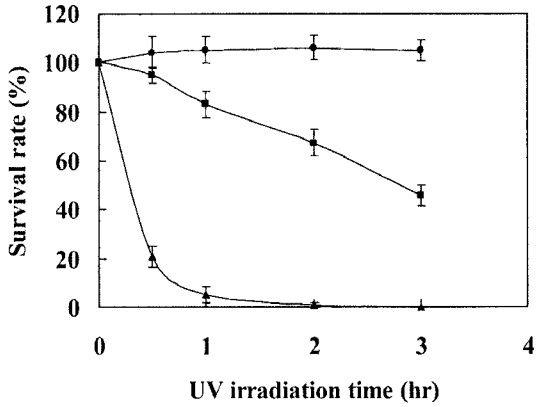

Fig. 4. Bactericidal effect of photocatalytic reactor on *L. monocytogenes*. Error bars represent standard deviations. (●, dark; ■, UV; ▲, UV+TiO_2)

Bactericidal activity of *L. monocytogenes* Another important pathogen present in certain lightly preserved food products is *L. monocytogenes*. Bactericidal effect of the photocatalytic reactor on *L. monocytogenes* was presented in Fig. 4. *L. monocytogenes* showed even less sensitivity to the photocatalytic reactor. Eighteen % of *L. monocytogenes* survived at 100 mL/min flow rate for 30 min. Complete sterilization was achieved after 3 hr of photocatalytic reaction.

In this study, a new approach using TiO_2 photocatalytic reactor has been applied successfully to examine TiO_2-mediated inactivation of food borne pathogenic bacteria. Continuous flow-sterilization system using photocatalytic reactor would be a useful design in diverse fields such as waste water treatment, and sterilization of aqueous foods. Waste water from the food industries and stock farms might have various microorganisms and organic compounds that may raise environmental contaminations. Although the sensitivities of microorganisms to the photocatalytic reactor may vary, it still has advantages that can decompose not only microorganisms but also other inorganic compounds at the same time. In case of the food industry, this continuous flow-sterilization system might also offer an effective way to sterilize aqueous foods without a thermal process. Furthermore, it might provide safe environment

for the operators without exposure to the UV lights. The use of TiO_2-coated alumina balls, in addition, provides the advantage that there is no need to filter to recover the TiO_2 powder. In this research, spherical alumina balls of 8-mm diameter were used in the photocatalytic reactor. The initial cell number of the bacteria used in this report was 10^8 CFU/mL. However in real food or water processing industries, the actual number of contaminated bacteria would be as low as under 10^2 CFU/mL. Therefore the reaction time that required achieving effective sterilization might be decreased. When the clarity of the liquid varies, the efficiency of this reactor may vary, of course, since the penetration of UV-A varies. Optimization of the reactor size and the diameter of the alumina balls should be made in real industries. Lots of systems that have flow of liquids such as food industries, oil refining industries, and water purifying systems have problem of fouling. To solve this problem additional steps are needed. The photocatalytic TiO_2 can decompose organic compounds. Due to this favorable characteristic of this reactor the formation of fouling might be reduced. On the other hand, variation of the diameter of the balls may influence the bactericidal activity due to the change of surface area and flow rate. Optimizations on the sizes of the balls and flow rate should be carried out depending to the objects.

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References

1. Sopyan I, Murasawa S, Hashimoto K, Fujishima A. Highly efficient TiO_2 film photocatalyst: Degradation of gaseous acetaldehyde. Chem. Lett. 23: 723-726 (1994)
2. Sauer ML, Ollis DF. Photocatalyzed oxidation of ethanol and acetaldehyde in humidified air. J. Catal. 158: 570-582 (1996)
3. Vorontsov AV, Savinov EN, Barannik GB, Troitsky VN, Parmon VN. Quantitative studies on the heterogeneous gas-phase photooxidation of CO and simple VOCs by air over TiO_2. Catal. Today 39: 207-218 (1997)
4. Wong HC, Chen MC, Liu SH, Liu DP. Incidence of highly genetically diversified *Vibrio parahaemolyticus* in seafood imported from Asian countries. J. Food. Microbiol. 52: 181-188 (1999)
5. Wills RW, Gray JT, Fedorka-Cray PJ, Yoon KJ, Scott L, Zimmerman JJ. Synergism between porcine reproductive and respiratory syndrome virus (PRRSV) and *Salmonella choleraesuis* in swine. J. Vet. Med. Sci. 71: 177-192 (2000)
6. Merrell DS, Andrew C. Acid tolerance of gastrointestinal pathogens. Curr. Opin. Microbiol. 5: 51-55 (2002)
7. Wang S, Ang HM, Tade MO. Volatile organic compounds in indoor environment and photocatalytic oxidation: State of the art. Environ. Int. 33: 694-705 (2007)
8. Praveena D, Swaminathan T. Novel photoreactors for heterogeneous photocatalytic wastewater treatment. Ann. Chim. 93: 805-809 (2003)
9. Sharma VK, Dutta PK, Ray AK. Review of kinetics of chemical and photocatalytical oxidation of arsenic (III) as influenced by pH. J. Environ. Sci. Heal. A 42: 997-1004 (2007)
10. Saien J, Neiati H. Enhanced photocatalytic degradation of pollutants in petroleum refinery wastewater under mild conditions. J. Hazard. Mater. 148: 491-495 (2007)
11. Hodgson AT, Destaillats H, Sullivan DP, Fisk WJ. Performance of ultraviolet photocatalytic oxidation for indoor air cleaning applications. Indoor Air 17: 305-316 (2007)
12. Fujishima A, Honda K. Electrochemical photocatalysis of water at semiconductor electrode. Nature 238: 27-38 (1972)
13. Wei C, Lin WY, Zainal Z, Williams NE, Zhu K, Kruzic AP, Smith RL, Rajeshwar K. Bactericidal activity of TiO_2 photocatalyst in aqueous media: Toward a solar-assisted water disinfection system. Environ. Sci. Technol. 28: 934-938 (1994)
14. Wilkins E, McDowell T, Pham HN. Photocatalytically-mediated disinfection of water using TiO_2 as a catalyst and spore-forming *Bacillus pumilus* as a model. J. Environ. Sci. Heal. A 30: 627-636 (1995)
15. Chun MJ, Shim E, Kho EH, Park KJ, Jung J, Kim JM, Kim BH, Lee KH, Cho DL, Bai, DH, Lee SI, Hwang HS, Ohk SH. Surface modification of orthodontic wires with photocatalytic TiO_2 for its anti-adherent and antibacterial properties. Angle Orthod. 77: 483-488 (2007)
16. Cai R, Kubota Y, Shuin T, Sakai H, Hashimoto K, Fujishima A. Induction of cytotoxicity by photo excited TiO_2 particles. Cancer Res. 52: 2346-2348 (1992)
17. Kubota Y, Shuin T, Kawasaki C, Hosaka M, Kitamura H, Cai R, Sakai H, Hashimoto K, Fujishima A. Photokilling of T-24 human bladder cancer cells with titanium dioxide. Brit. J. Cancer 70: 1107-1111 (1994)
18. Ireland JC, Klostermann P, Rice EW, Clark RM. Inactivation of *Escherichia coli* by titanium dioxide photocatalytic oxidation. Appl. Environ. Microb. 59: 1668-1670 (1993)
19. Matsunaga T, Tomada R, Nakajima T, Wake H. Photochemical sterilization of microbial cells by semiconductor powders. FEMS Microbiol. Lett. 29: 211-214 (1985)
20. Kim BH, Kim DH, Cho DL, Cho SY. Bactericidal effect of TiO_2 photocatalyst on selected food-borne pathogenic bacteria. Chemosphere 52: 277-281 (2003)