Sterilization of Pathogenic Bacteria Using Titanium Dioxide Photocatalyst

Tae-Young Kim[a,b,], Kyung-Hee Park[b], Tae-Gwang Jeoung[a,b], Seung-Jai Kim[a,b,c], Sung-Yong Cho,[a,b]*

[a]Department of Environmental Engineering, Chonnam National University, Gwangju 500-757, Korea

[b]BK 21 Development of environmental friendly core materials and processes
[c]Environmental Research Institute, Chonnam National University, Gwangju 500-757, Korea

Abstract. Titanium dioxide (TiO_2) photocatalysts have attracted great attention as a material for photocatalytic sterilization in the food and environmental industry. This research aimed to design a new photobioreactor and its application to sterilize selected pathogenic bacteria, *Salmonella choleraesuis subsp., Vibrio parahaemolyticus, Listeria monocytogenes,* and *Pseudomonas sp*. The photocatalytic reaction was carried out with various TiO_2 concentrations, ultraviolet (UV) illumination time.

The bactericidal effect of TiO_2 under UV light irradiation on all bacterial suspension was much higher than that of without TiO_2. As the concentration of TiO_2 increased to 1.0 mg/ ml, bactericidal effect increased. The complete killing time of *Salmonella, Vibrio and Pseudomona*s was achieved after 3 hr but *Listeria* was more resistant. However, titanium dioxide powder could not be used satisfactorily for the sterilization of the bacteria due to its poor separation from the suspension. Therefore, in this study, chitosan bead coated with titanium dioxide was made by sol-gel method, and used for sterilization. The survival ratio of *Salmonella* using chitosan bead coated with TiO_2 was greater than that of chitosan bead.

Keywords: Titanium dioxide, *Salmonella, Vibrio, Listeria, Pseudomonas*, chitosan.
*To whom correspondence should be addressed: e-mail: tykim001@chonnam.ac.kr

INTRODUCTION

TiO_2 (Titanium oxide) photocatalysts have attracted great attention as alternative materials to aids in the purification of water and air.[1-3] TiO_2 photocatalysts generate strong oxidizing power when illuminated with UV light with wavelength of less than 385nm. 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 water.

Wastewater from hospital, food factories, and contaminated sites sometimes contains microorganisms, virus, and organic compounds. One of the typical sterilization method is the photocatalytic sterilization by illumination of UV light with the wavelength of 254nm, which provides a high rate of sterilization at room temperature. Alternately, it is well known that the TiO_2 in anatase form is capable of oxidizing and the decomposing various kinds of compounds.[3-5]

Salmonella choleraesuis are 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 syndrom (PRRS) as a new swine disease have both occurred only relatively recently.[6] *Vibrio parahaemolyticus* is a prevalent food-borne pathogen in many Asian countries where marine foods are frequently consumed. Environmental strains of *V. parahaemolyticus* are typically not human pathogens. However, these strains cause disease in shrimps,

oysters, mussels and other marine invertebrates and in fishes. *Listeria monocytogenes* is a Gram-positive facultative intracellular pathogen that is the causative agent of food borne listeriosis.[7]

TiO_2 particles catalyze the killing of bacteria and cancer cells[8] by 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.[9] In 1985, Matsunaga and coworkers 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 120min. Since then, research work on TiO_2 photocatalytic killing has been intensively conducted on a wide spectrum of organisms including viruses, bacteria, fungi, algae, and cancer cells.

For practical applications, however, there are some difficulties such as either filtration of fine TiO_2 or fixation of catalyst particles. For these reasons, many scientists have being working to increase the efficiency of this process. One of direction is to improve efficiency of TiO_2 by modification of its surface. TiO_2 supported on the porous materials such as SiO_2, ZrO_2, zeolites and activated carbons were used to increase the efficiency. It was also reported that the addition of activated carbon to TiO_2 slurry could increase decomposition of some organic compounds during the photocatalytic process.[10] It has an additional benefit in reducing the recombination rate of electron with hole by increasing the energy of the band gap in TiO_2. In other papers[11], carbon coating of photocatalytic TiO_2 particles was successfully performed without pronounced interruption of photoactivity. Much higher photocatalytic performance was obtained because of notable suppression of phase transformation to less photoactive rutile and also acceleration of crystal growth of anatase phase.

In this study, sterilization experiments for *S. choleraesuis* subsp., *Vibrio parahaemolyticus, Listeria monocytogenes, Pseudomonas sp* were conducted in a batch type photocatalytic reactor in terms of UV illumination time, TiO_2 concentration. And TiO_2 was coated on chitosan bead for the easy recovery of the photocatalyst after photocatalytic-reaction.

MATERIALS AND METHODS

Two crystalline forms of TiO_2 have photocatalytic activity, anatase and rutile. Anatase has a band gap of 3.2 eV and for rutile it is 3.0 eV. Anatase has been found to be the most active form. The action spectrum for anatase shows a sharp decrease in activity above about 385 nm. The photocatalytic process includes chemical steps that produce reactive species that in principal can cause fatal damage to microorganisms. The reactive oxygen species (ROS) may disrupt or damage various cell or viral functions or structures. The preponderance of evidence on photocatalytic chemistry in aqueous solution suggests that the hydroxyl radical formed by hole transfer does not diffuse from the surface of the TiO_2 into bulk aqueous phase[13]. For a cell or virus in contact with the titanium dioxide surface there also be direct electron or hole transfer to the organism or one of its components. If titanium dioxide particles are of small size, they may penetrate into the cell and these processes could in the interior. Since light is an essential component of the photocatalytic system, there can also be direct photochemistry as there would be from any UV source. Mechanism of a photocatalytic process on irradiated titanium dioxide:

Electron-Hole Pair Formation

$$TiO_2 + hv \rightarrow TiO_2^{-} + OH^{\bullet} \text{ (or } TiO_2^{+}\text{)} \quad (1)$$

[conduction band electron and valence band hole]

Electron removal from the conduction band

$TiO_2^- + O_2 + H^+ \rightarrow TiO_2 + HO_2$ (2)

$TiO_2^- + H_2O_2 + H^+ \rightarrow TiO_2 + H_2O + OH^\bullet$ (3)

$TiO_2^- + H^+ \rightarrow TiO_2 + \bullet H_2$ (4)

Oxidation of organic compounds

$OH^\bullet + O_2 + C_nO_mH_{(2n-2m+2)} \rightarrow nCO_2 + (n-m+1)H_2O$ (5)

Nonproductive Radical Reactions

$TiO_2^- + OH^\bullet + H^+ \rightarrow TiO_2 + H_2O$ [recombination] (6)

$2OH^\bullet \rightarrow H_2O_2$ (7)

$2HO_2^- \rightarrow H_2O_2 + O_2$ (8)

$OH^\bullet + H_2O_2 \rightarrow H_2O + O_2$ (9)

$OH^\bullet + HCO_3^- \rightarrow CO_3^{\bullet -} + H_2O$ (10)

Hydroxyl radicals are highly reactive and therefore short-lived. Superoxide ions are more long-lived; however, due to the negative charge they cannot penetrate the cell membrane. Upon their production on the TiO_2 surface, both hydroxyl radicals and superoxide would have to interact immediately with the outer surface of an organism unless the TiO_2 particle has penetrated into the cell.

Compared to hydroxyl radicals and superoxide ions, hydrogen peroxide is less detrimental. However, hydrogen peroxide can enter the cell and be activated by ferrous ion via the Fenton reaction.

$Fe^{+2} + H_2O_2 \rightarrow OH^\bullet + OH^{-1} + Fe^{+3}$ (11)

The ability of bacteria, such as *E. coli*, to sequester iron is well documented. Iron levels on the cell surface, in the periplasmic space or inside the cell, either as iron clusters or in iron storage proteins (such as ferritin) are significant and can serve as a source of ferrous ion. Therefore, while the TiO_2 is being illuminated to produce H_2O_2, the Fenton reaction may take place *in vivo* and produce the more damaging hydroxyl radicals. When the light is turned off, any residual hydroxyl radicals through the Fenton reaction. When both H_2O_2 and superoxide ion are present, the iron-catalyzed Haber-Weiss reaction can provide a second pathway to form additional hydroxyl radicals.

$Fe^{+3} + O_2^- \rightarrow Fe^{+2} + O_2$ (12)

$Fe^{+2} + H_2O_2 \rightarrow Fe^{+3} + OH^- + OH^\bullet$ (13)

Since the initial actions of these reactive oxygen species (ROS) target the outer surface of a cell, the rigidity and chemical arrangements of their surface structure will determine how effectively the TiO_2 photocatalytic disinfection process functions.

TiO_2

The photocatalyst TiO_2 (anatase) was obtained from Aldrich Co.. The physical properties of TiO_2 were characterized by BET analysis. The specific surface area, pore size and pore volume were 12.02 m_2/g, 57.48 Å and 0.0172 cm^3/pore, respectively. Prior to use, this compound was sealed and stored at

room temperature.

CHTOSAN BEAD COATED WITH TiO_2

The supports for TiO_2 powder used in this study were chitosan, manufactured by Sehwa Co. (South Korea). Chitosan was obtained as flaked type from Sehwa Co., without further purification. Flaked chitosan was milled to pass through a 180-μ m sieve. The degree of deacetylation of chitosan flakes was determined to be 85%. The average molecular weight of the chitosan was measured to be 8.2×10^5 by the Mark–Houwink equation from viscosity measurements at different concentrations. When used as a support for TiO_2 powder, the powdered chitosan was dissolved in a 2 wt% acetic acid solution to produce a viscous solution with approx. 2 wt% chitosan. TiO_2 powders (5 g) were dispersed into 100 ml of the chitosan solution during 24 h under continuous stirring. Thereafter, the mixed solution was cast into beads by a phase-inversion technique using 2 M NaOH. The physical properties of the prepared chitosan bead and TiO_2-coated chitosan beads are given in Tables 1.

Table 1. Physical properties of chitosan bead.

	Unit	Chitosan bead	Chitosan beads coated with TiO_2
Particle diameter	㎜	2.0	2.0
Multi point BET	㎡/g	350	79
Average pore diameter	Å	856	97.4

MICROORGANISMS AND CULTURE CONDITIONS

Microorganisms, *Salmonella choleraesuis subsp*., *Vibrio parahaemolyticus, Listeria monocytogenes, Pseudomonas sp.* were purchased from KCCM (Korean Culture Collection of Microorganisms, Seoul, Korea). The culture medium for stock cultures of *S. choleraesuis subsp., V. parahaemolyticus, L. monocytogenes, Pseudomonas sp.* was typtic soy broth (TSB, Merck, Germany), TSB with 3% (w/v) NaCl, and a brain heart infusion (BHI, Becton Dickinson, USA) respectively. The working culture was prepared by transferring each bacterium from stock culture to 300 ml conical glass flasks containing 50 ml of medium. Culture flasks were aerobically incubated on a rotary shaker at 33^{o}C for 24 h.
Planktonic cell preparation *S. choleraesuis subsp., L. monocytogenes V. parahaemolyticus and Pseudomonas sp.* cells grown from working culture were harvested by centrifugation of them using a centrifuge (Hanshin, medical, Co., LTD., Korea) at 4000 rpm for 15 min. *S. choleraesuis subsp*. L. monocytogenes and *Pseudomonas sp.* were washed twice using a sterile phosphate-buffer saline (PBS, pH 7.2) and resuspended with PBS. The cells of *V. parahaemolyticus* was washed twice in sterile deionized water containing 3% (w/v) NaCl. All the washed cells in solution was counted and stored at 5 ^{o}C until use.

PHOTOCATALYTIC REACTION

The photocatalytic reactor consisted of UV lamp (A), sampling tube (B), magnetic stirrer (C), air filter (D), thermo-circulator (E), peristatic pump (F), sampling tube (G), and temperature controller (H) (Fig. 1). The reaction was performed at 1000 ml working volume of batch reactor.

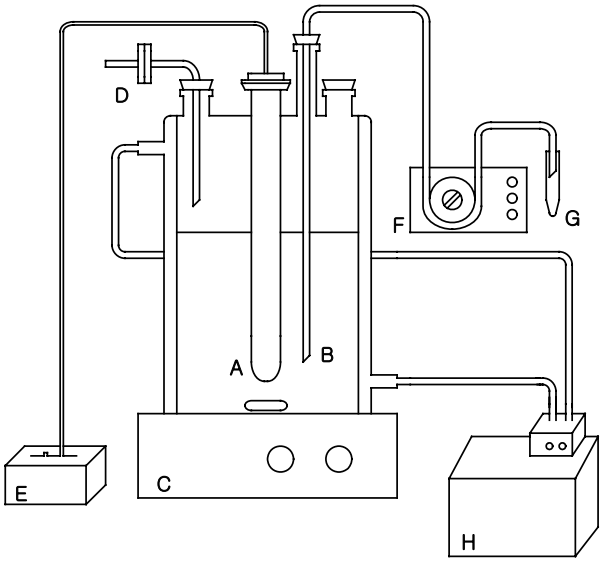

Fig. 1. Experiment apparatus for photocatalytic reaction (Batch reactor system). A: UV lamp, B: Sampling tube, C: Stirrer, D: Air filter, E: UV power supply, F: Peristatic pump, G: Sample tube, H: Temperature controller

UV lamp was centered to illuminate properly the inner of vessel while the mixture of cells and TiO_2 were agitated continuously. Temperature was controlled 25℃ using the thermo-circulator during the photocatalytic reaction. The initial concentration of cells of *S. choleraesuis* subsp., *V. parahaemolyticus, L. monocytogenes* and *Pseudomonas sp.* was adjusted to about 10^8 cfu/ml. To investigate the optimal dose of TiO_2, photocatalytic reactions were carried out with TiO_2 concentrations ranging from 0.25 to 1.25 mg/ml. The control was treated either without TiO_2 or with UV only. The illumination time was 3 hr for *S. choleraesuis* subsp., *V. parahaemolyticus, L. monocytogenes* and *Pseudomonas sp.* For vial cell counting, the media of *S. choleraesuis* subsp., *V. parahaemolyticus, L. monocytogenes* and *Pseudomonas sp.* was a TSA respectively. Bacterial cells were sampled in triplicates at every 30 min and 1 hr for 3 hr. All plates were incubated at 33℃ for 24 h.

EFFECT OF UV ILLUMINATION TIME AND TiO_2 CONCETRATION

The photoreactor employed in batch process was designed for the mixture of cells and TiO_2 to contact continuously or be close to the surface of UV lamp as much as possible. In all cases, the inactivation of cells was evaluated by counting the viable cells with regular sampling. The feasible synergistic effect by UV and TiO_2 after 1 h or more treatments was appeared in all three strains. (Fig. 2: *S. choleraesuis subsp*., Fig. 3: *V. parahaemolyticus*, Fig. 4: *L. monocytogenes*, Fig. 5: *Pseudomonas sp.*). These Figures showed the changes in the cells viabilities of S. choleraesuis subsp. (Fig. 2), V. parahaemolyticus (Fig. 3), L. monocytogenes (Fig. 4) and *Pseudomonas sp.*(Fig. 5), respectively during constant UV illumination at various TiO_2 concentrations ranging from 0 to 1.25 mg/ml. After 30 min of irradiation with UV light in the presence of 1 mg TiO2/ml, 62% of S. choleraesuis subsp. lost survival. However, when the concentration of TiO_2 was decreased to 0.25 mg/ml, killing efficiency was decreased to 55% of bacteria. TiO_2 concentrations greater than 1 mg/ml resulted in a decrease in killing efficiency. We supposed that probably this trend related to be so many TiO_2 particles that the intensity of UV illumination became weak. As a result, the most effective TiO_2 concentration in the present study was 1 mg/ml. In the case of 1 mg/ml TiO_2 concentration, complete killing time was observed at 3 h and more than 99% of the cells lost their viability. Almost complete killing was achieved after 2 h of illumination. After 30 min of irradiation with UV light in the presence of 1 mg TiO_2/ml, more than 80% of V. parahaemolyticus lost viability (Fig. 3B). When the concentration of TiO_2 was decreased to 0.25 mg/ml, killing efficiency was a decrease to 35% of bacteria. Similar as S. choleraesuis subsp., TiO_2 concentrations greater than 1 mg/ml resulted in a decrease in killing efficiency. As a result, the most effective TiO_2concentration in the present study was 1 mg/ml. In the case of 1 mg/ml TiO_2 concentration, complete killing time was observed at about 2 h and more than 99% of the cells lost their viability. The survival curve of L. monocytogenes in Fig. 3B indicated that killing activity was much lower than that of the other bacteria. At 30 min illumination, only 35% of cells were killed and their complete killing did not appear until 4 h reaction. In this study, a sensitive approach using TiO_2 has been adapted successfully to examine TiO_2-mediated damages and their contribution to the deactivation of viable cells.

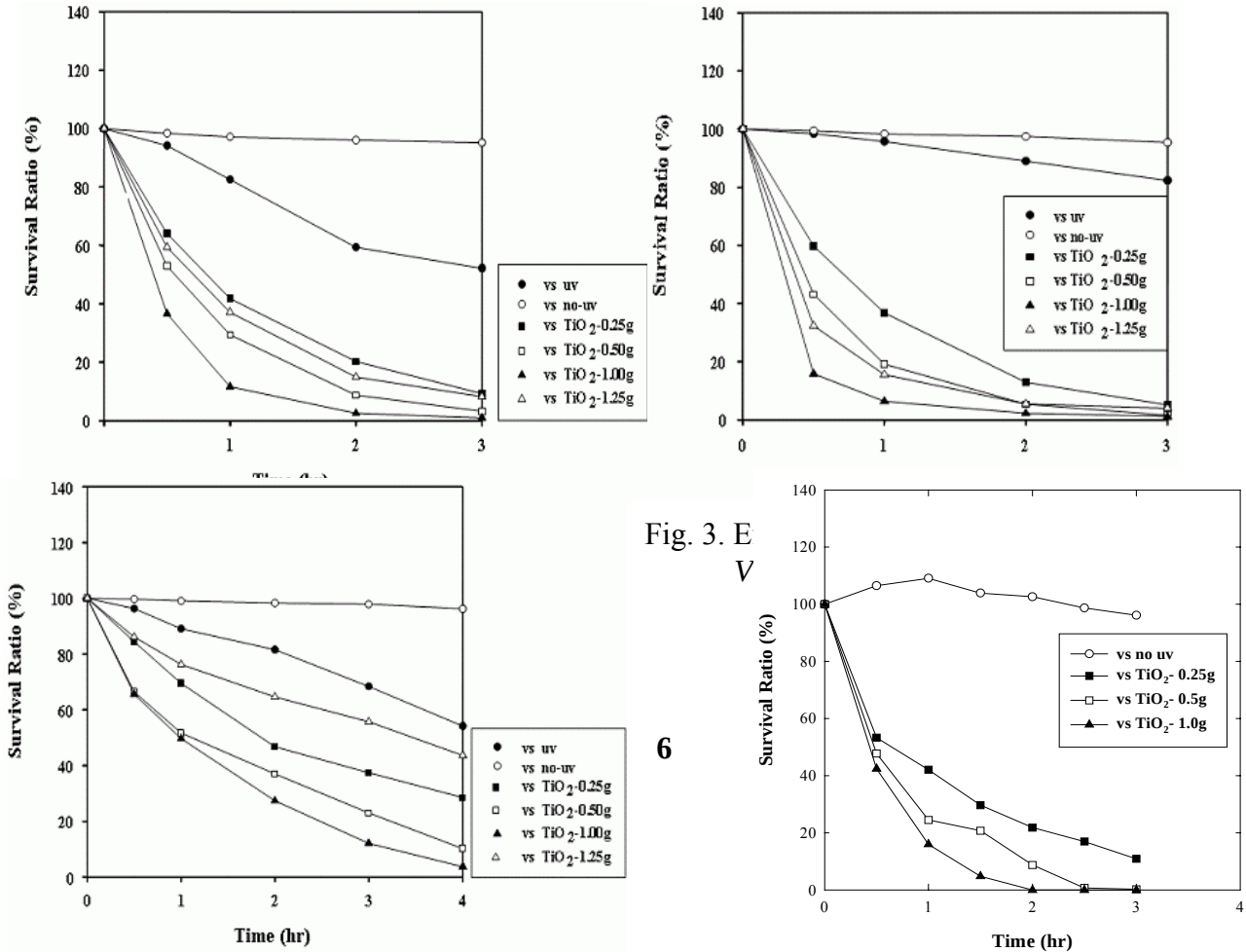

Fig. 3. E
V

Fig. 4. Effect of TiO_2 dose on the survival ratio of *L. monocytogenes* in the batch reactor.

Fig. 5. Effect of TiO_2 dose on the survival ratio of *Pseudomonas* sp. in the batch reactor.

Data from Figs. 2–5 suggest that TiO_2 particles remaining in the slurry may still retain their bactericidal activity. Another explanation is that certain lethal reactions would continue to propagate even after the UV illumination stops. This effect may be masked by the standard viable count procedure involving serial dilutions in buffered saline, as it allows cells to form colonies on rich nutrient media, which in turn allows the injured cell to recover. It is not surprising that cells would lose viability even after the removal of TiO_2 and/or UV-light.

.

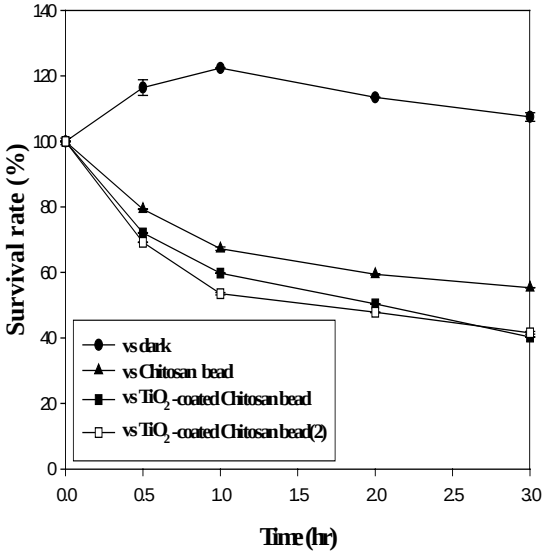

Fig. 6. Comparison of the survival ratio of *S.choleresuis* subsp. for chitosan bead and chitosan bead coated TiO_2 in the batch reactor.

Fig. 6 shows the bactericidal effect of S. choleraesuis subsp for chitosan bead and TiO_2 coated on chitosan bead. As can been seen in this figure, the survival ratio of S. choleraesuis subsp onto chitosan bead is lower than that of TiO_2 coated on chitosan bead. Furthermore, the similar results were obtained when we processed the experiment with recycled TiO_2-coated chitosan bead. We have examined the possibility of use chitosan bead as a support material for TiO_2 through this experiment

Titanium dioxide (TiO_2) powder could not have been used satisfactorily due to its poor recovery in water contain bacteria. However, TiO_2 coated on chitosan bead can be applied to environment purification.

CONCLUSIONS

In the study of the photocatalytic bactericidal effect of TiO_2 on food pathogenic bacteria, it was confirmed that both near-UV illumination time and TiO_2 concentrations influenced the cell killing

activity. We founded that optimal TiO_2 concentration was 1mg TiO_2/ml. After 30 min near-UV illumination, killing activity of V. parahaemolyticus cells was higher than other bacteria, however, L. monocytogenes cells showed a lower killing activity than that of other bacteria.

Killing activity of *S.choleresuis* subsp for TiO_2 coated on chitosan bead was more efficient than chitosan bead. Furthermore, the similar results were obtained when we processed the experiment with recycled TiO_2-coated chitosan bead. We have examined the possibility of use chitosan bead as a support material for TiO_2 through this experiment.

ACKNOELEDGEMENT

This research was financially supported by the Brain Korea 21

REFERENCES

1. Meacock, G., Taylor, K.D.A., Knowles, M., Himonides, A., (1997), The improved whitening of minced cod flesh using dispersed titanium dioxide, *J. Sci. Food Agric*. **73**, 221-225.
2. Burrows, H., Ernestova, L., Kemp, T., Skurlatov, Y., Purmal, A., Yermankov, A., (1998), Kinetics and mechanism of photodegradation of chlorophenols, *Prog. Reaction Kinetics* **23**, 145-151.
3. Turkoglu, M., Yener, S., (1997), Design and in vivo evaluation of ultrafine inorganic-oxide-containing-sunscreen formulation, *Int. J. Cosmetic. Sci.* **19**, 193-201.
4. Sauer, M.L., Ollis, D.F., (1996), Photocatalyzed oxidation of ethanol and acetaldehyde in humidified air. *J. Catal*. **158**, 570-582.
5. Sturini, M., Fasani, E., Prandi, C., Casaschi, A., Albini, A., (1996), Titanium dioxide-photocatalysed decomposition of some thiocarbamates in water, J. Photochem. Photobiol. *A: Chem*. **101**, 251-255.
6. Wang, L.L., Johnson, E.A., (1992), Inhibition of *Listeria monocytogenes* by fatty acids and monoglycerides. *Appl. Environ. Microbiol*. **58**, 624-629.
7. Matsunaga, T., Tomoda, R., Nakajima, T., Wake, H., (1985), Photoelectrochemical sterilization of microbial cells by semiconductor powers, FEMS Microbiol. Let. **29**, 211-214.
8. Pham, H.N., McDowell, Wikins, T., E., (1995), Photocatalytically-mediated disinfection of water using TiO_2 as a catalyst and spore-forming *Bacillus pumilus* as a model, *J. Environ. Sci. Health* **A30 3**, 627-636.
9.. Wills, R.W., Gray, J.T., Fedorka-Cray, P.J., Yoon, K.J., Scott Ladely., Zimmerman, J.J., (2000), Synergism between porcine reproductive and respiratory syndrome virus (PRRSV) and *Salmonella choleraesuis* in swine. *J. Vet. Med. Sci*. **71**, 177-192.
10. Cai, R., Kubota, Y., Shuin, T., Sakai, H., Hashimoto, K., Fujishima, A., (1992), Induction of cytotoxicity by photo excited TiO_2 particles, *Cancer Res*. **52**, 2346-2348.
11. Kubota, Y., Shuin, T., Kawasaki, C., Hosaka, M., Kitamura, H., Cai, R., Sakai, H., Hashimoto, K., Fujishima,A., (1994), Photokilling of T-24 human bladder cancer cells with titanium dioxide, Br. *J. Cancer,* **70**, 1107-1111.
12. Ireland, J.C., Klostermann, P., Rice, E.W., Clark, R.M., (1993), Inactivation of *Escherichia coli* by titanium dioxide photocatalytic oxidation. *Appl. Environ. Microbiol*. **59**, 1668-1670.

13. Fox, M.A., Dulay, M.T., (1993), Heterogeneous photocatalysis. *Chem. Rev*. **93**, 341-357.