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Linear correlation between inactivation of *E. coli* and OH radical concentration in TiO_2 photocatalytic disinfection

Min Cho[a], Hyenmi Chung[b], Wonyong Choi[c], Jeyong Yoon[a,*]

[a] *School of Chemical Engineering, College of Engineering, Seoul National University, San 56-1, Sillim-dong, Gwanak-gu, Seoul 151-742, South Korea*

[b] *Water Microbiology Division, National Institute of Environmental Research, Kyungseo-dong, Seo-gu, Inchon 404-170, South Korea*

[c] *School of Environmental Science and Engineering, Pohang University of Science and Technology, Pohang 790-784, South Korea*

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Abstract

The biocidal action of the TiO_2 photocatalyst has been now well recognized from massive experimental evidences, which demonstrates that the photocatalytic disinfection process could be technically feasible. However, the understanding on the photochemical mechanism of the biocidal action largely remains unclear. In particular, the identity of main acting photooxidants and their roles in the mechanism of killing microorganisms is under active investigation. It is generally accepted that reactive oxygen species (ROS) and OH radicals play the role. The aim of this study is to determine how the OH radical, acting either independently or in collaboration with other ROS, is quantitatively related to the inactivation of *E. coli*. The steady-state concentrations of OH radicals ($[\bullet OH]_{ss}$) in UV-illuminated TiO_2 suspensions could be quantified from the measured photocatalytic degradation rates of *p*-chlorobenzoic acid (a probe compound) and its literature bimolecular rate constant with OH radicals. The results demonstrated an excellent linear correlation between $[\bullet OH]_{ss}$ and the rates of *E. coli* inactivation, which indicates that the OH radical is the primary oxidant species responsible for inactivating *E. coli* in the UV/TiO_2 process. The *CT* value of OH radical for achieving 2 log *E. coli* inactivation was initially found to be 0.8×10^{-5} mg min/l, as predicted by the delayed Chick–Watson model. Although the primary role of OH radicals in photocatalytic disinfection processes has been frequently assumed, this is the first quantitative demonstration that the concentration of OH radicals and the biocidal activity is linearly correlated.

Keywords: TiO2; *E. coli*; Photocatalysis; OH radical; ROS (reactive oxygen species)

1. Introduction

Since the photochemical sterilization of *E. coli* using Pt-TiO_2 was first reported by Mutsunaga et al. [1], many photocatalytic disinfection studies using TiO_2 have been carried out, not only to determine the major reaction parameters (TiO_2 concentration, light intensity and pH, etc.), but also to improve our understanding of the disinfection mechanism responsible for the inactivation of the microorganism [2–6]. Although a wealth of information has been amassed, which demonstrates the efficacy of the biocidal actions of the TiO_2 catalyst, there is a lack of any definite evidences regarding the major species which is responsible for the photochemical inactivation of the microorganism. The biocidal action of the TiO_2 photocatalyst has been frequently ascribed to two major photochemical oxidants: OH radical [3,4,7–11] and reactive oxygen species (ROS) [12,13]. Out of several published experimental evidences in this regard, Ireland et al. [10] reported no inactivation of *E. coli* in the presence of the OH radical scavenger. More definitively, Sjogren and Sierka [9], in their study

*Corresponding author. Tel.: +82-2-880-8927; fax: +82-2-876-8911.

E-mail address: jeyong@snu.ac.kr (J. Yoon).

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of MS-2 phage inactivation involving the photocatalytic TiO_2 reaction, reported a 200% enhancement of its inactivation in the presence of a low concentration of ferrous ions. This observation of enhanced MS-2 phage inactivation was explained by the increased OH radical concentration resulting from the Fenton reaction, as well as from the photocatalytic reaction ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + \bullet OH$, $Fe^{3+} + e^-_{eb} \rightarrow Fe^{2+}$) which was facilitated in the presence of the added ferrous ions.

ROS includes not only the OH radical, but also $O_2\bullet^-$, and H_2O_2 [2,12,13]. Kikuchi et al. [2] proposed that the main bactericidal source is not the OH radical, but H_2O_2 in *E. coli* suspension separated from the TiO_2 surface by a porous PTFE membrane, since catalase that can decompose H_2O_2 greatly hindered the inactivation of *E. coli*, whereas the OH radical scavenger did not. With this observation, they proposed that the inactivation mechanism consisted of H_2O_2 and $O_2\bullet^-$ entering the inner cell through a process of diffusion, and subsequently generating the OH radical by means of the Harber–Weiss reaction ($O_2\bullet^- + H_2O_2 \rightarrow \bullet OH + OH^- + O_2\bullet$). However, the facts that H_2O_2 formation (2×10^{-7} M) is limited in the TiO_2 suspension and that the rate constant of the Harber–Weiss reaction is very low ($k < 1\,M^{-1}s^{-1}$) do not support this mechanism [2]. On the other hand, Maness et al. [13] reported that *E. coli* inactivation was caused by ROS as a result of cell wall damage and subsequent peroxidation of the polyunsaturated phospholipid component of the lipid membrane. However, they did not cite any specific agent responsible for *E. coli* inactivation among the different ROS.

This study was conducted to determine how the OH radical, acting either independently or in collaboration with other ROS, is related to the inactivation of the microorganism. *E. coli* was used as a model microorganism. The *CT* values of OH radical responsible for microorganism inactivation were quantitatively investigated at a certain level of *E. coli* inactivation. The OH radical concentration under a specific experimental condition could be controlled or measured using either an OH radical scavenger or an OH radical probe compound. The quantified OH radical concentrations were correlated with the activity of *E. coli* inactivation.

2. Materials and methods

2.1. Materials

All solutions and reagents were prepared in deionized/distilled water, which was supplied by a Barnstead NANO pure system (Barnstead, USA). Analytical reagent-grade chemicals were used (Aldrich, USA). All glassware used in these experiments was washed with distilled water, and then autoclaved at 121°C for 15 min. Degussa P25 TiO_2 was used as a photocatalyst, which was suspended in water with 30 min of pre-sonication to disperse the particles uniformly.

2.2. Experimental procedures

The disinfection experiments were carried out in a 60 ml Pyrex reactor (UV cut-off <300 nm) with 50 ml of solution and the scheme of experimental apparatus was described in Fig. 1. The slurry of TiO_2/*E. coli* was intensively mixed with a magnetic stirrer (EYELA Co., RC-2, Japan) with a speed of 1100 rpm to allow a complete mixing. Five sets of photocatalytic disinfection experiments were carried out with varying the parameters of (1) dissolved O_2 or OH radical scavenger, (2) TiO_2 concentration, (3) light intensity, (4) temperature, and (5) pH. Under the different experimental conditions, different steady-state concentrations of OH radicals should result with different activities of *E. coli* inactivation. Two types of oxic conditions for the photocatalytic disinfection experiments were tested in this study: air equilibration with magnetic stirring or O_2 saturation with continuous oxygen sparging with the flow rate of 0.2 ml/min. The dissolved oxygen concentration was reduced from 8.0 to 3.5 mg/l at the end of the experiments under the condition of air equilibration. An anoxic environment was obtained by sparging nitrogen gas. Dissolved oxygen was measured with a DO Meter (YSI 710A, USA). The TiO_2 concentrations ranged from 0.1 to 2.0 g/l. The light source was black light blue lamps (BLB 18 W, Philips) (four or fewer) which emit in the 300–420 nm range. The lamps were positioned inside the reactor, with one being placed on each side. The emission spectrum of the BLB lamp was measured with an Acton Research Detection system (Spectrapro-500, USA). The light intensity, which was measured by ferrioxalate actinometry [14], varied from 1.5×10^{-6} to 7.9×10^{-6} Einstein/l s at 20°C. The reaction temperature was controlled with a thermostatic chamber (Jeio Tech, Korea), whose temperature was varied from 6°C to 30°C. Though the same experimental

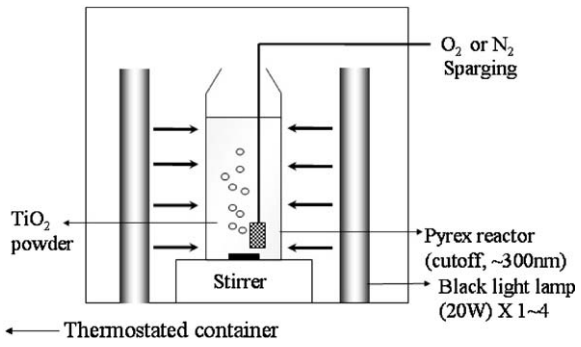

Fig. 1. The scheme of TiO_2 experimental apparatus.

apparatus was used, because diffusion efficacy of lamp itself might be influenced by environmental temperature, light intensity was measured each temperature, 6°C and 33°C as 6.2×10^{-6} to 8.7×10^{-6} Einstein/l s, respectively. The pH was adjusted to 5.6 or 8.2 using a phosphate buffer (KH_2PO_4/NaOH). The resulting phosphate buffer solutions were maintained at approximately 20 mM. *E. coli* (ATCC strain 8739), with initial concentrations 10^5–10^6 cfu/ml, was chosen as the indicating microorganism. No inactivation of *E. coli* was observed in the absence of either BLB radiation or TiO_2 particles within the present experimental time scale.

Table 1 provides details of all the experimental conditions employed in this study. Experiments No. 1–6, No. 7–9, No. 10 and 11, No. 12 and 13, and No. 14 and 15 were performed to examine the effect of oxygen and the OH radical scavenger, TiO_2 concentration, light intensity, temperature, and pH on the inactivation of *E. coli*, respectively. The disinfection experiments from No.1 to No. 6 were repeated three times to confirm their reproducibility.

2.3. Analysis and culture of bacteria

E. coli was inoculated in Tryptic Soy Broth in a 50 ml/200 ml flask and grown for 18 h at 37°C. The bacteria were harvested by centrifugation in a 50 ml conical tube at 1000 g for 10 min and washed two more times with 50 ml phosphate buffered saline solution (pH 7.2, Sigma P 0261, USA). *E. coli* stock solution was prepared by resuspending the final pellets in 50 ml of phosphate buffered saline solution. The initial populations of *E. coli* ranged approximately from 10^4 to 10^5 cfu/ml and were obtained by diluting the stock solution. The cell concentration was determined by the spread plate method with nutrient agar grown at 37°C for 24 h. One milliliter of solution was withdrawn at each sampling and was diluted to 1/1, 1/10 and 1/100. Finally, 0.1 ml of the undiluted and diluted solutions was spread to count the number of *E. coli*. Three replicate plates were used at each dilution.

2.4. Analysis of pCBA

The concentration of *p*-chlorobenzoic acid (*p*CBA, 1.92 μM), the OH radical probe compound used in this study, was analyzed with an HPLC analytical system (Waters Co., 1525 Binary system, USA). A C_{18} reverse-phase column (XTerra Rp-18 reverse-phase column (5 μm, 150 mm × 2.1 mm)) was used with a UV detector (Gilson Co., 151 UV/VIS, USA) at 230 nm for measuring the *p*CBA concentration. A solvent mixture of 35% acetonitrile/65% water containing 40 mM phosphate buffer was used as the mobile phase.

The degradation reaction of *p*CBA, which was chosen as a probe for OH radical formation, can be expressed by Eq. (1):

$$-\frac{d[pCBA]}{dt} = k_{exp}[pCBA] = k_{OH,pCBA}[\bullet OH]_{ss}[pCBA]. \quad (1)$$

Integration of Eq. (1) yields:

$$-\ln\frac{[pCBA]}{[pCBA]_0} = k_{exp}t, \quad (2)$$

where $k_{exp} = k_{OH,\ pCBA}[\bullet OH]_{ss}$.

Table 1
List of the experimental conditions employed in this study

Exp. ID	TiO_2 (g/l)	Light intensity (Einstein/l s)	Temp. (°C)	pH	MeOH	Gas sparging	No. of exp. set[a]	No. of samples	k_{exp} (s^{-1})
1	1.0	7.9×10^{-6}	20	7.1	—	N_2	3	12	0.000 ± 0.002[b]
2	1.0	7.9×10^{-6}	20	7.1	—	—	3	18	0.020 ± 0.001
3	1.0	7.9×10^{-6}	20	7.1	—	O_2	3	18	0.031 ± 0.003
4	1.0	7.9×10^{-6}	20	7.1	O	N_2	3	12	0.000 ± 0.001
5	1.0	7.9×10^{-6}	20	7.1	O	—	3	12	0.000 ± 0.001
6	1.0	7.9×10^{-6}	20	7.1	O	O_2	3	12	0.000 ± 0.000
7	0.1	7.9×10^{-6}	20	7.1	—	—	1	7	0.010 ± 0.001
8	0.5	7.9×10^{-6}	20	7.1	—	—	1	8	0.014 ± 0.002
9	2.0	7.9×10^{-6}	20	7.1	—	—	1	6	0.021 ± 0.001
10	0.5	1.5×10^{-6}	20	7.1	—	—	1	6	0.006 ± 0.000
11	0.5	3.4×10^{-6}	20	7.1	—	—	1	4	0.008 ± 0.001
12	1.0	6.2×10^{-6}	6	7.1	—	—	1	6	0.015 ± 0.003
13	1.0	8.7×10^{-6}	33	7.1	—	—	1	6	0.024 ± 0.004
14	1.0	7.9×10^{-6}	20	5.7	—	—	1	6	0.021 ± 0.001
15	1.0	7.9×10^{-6}	20	8.2	—	—	1	6	0.021 ± 0.002

[a] No. of exp. set, number of independent experimental set.
[b] Standard deviation of *p*CBA degradation rate constant obtained at each of the three stages, the initial, middle and final stage.

The steady-state OH radical concentration ($[\bullet OH]_{ss}$) can be calculated from Eq. (1) ($k_{OH,pCBA} = 5 \times 10^9\,M^{-1}\,s^{-1}$, [15]). The observed degradation rate constant of *p*CBA (k_{exp}) can be obtained from the slope of the semi-log plot of *p*CBA degradation, as shown in Eq. (2). In all cases, *p*CBA degradation experiments at each disinfection experiment were conducted at three different reaction periods, the initial, middle and final stage of the disinfection experiments. These multiple *p*CBA degradation experiments at each disinfection experiment were done since one *p*CBA degradation experiment does not represent the status of OH radical concentration during the whole duration of each disinfection experiment. The *p*CBA degradation data obtained at each of the three stages of each disinfection experiment were almost the same, within a 95% confidence interval, indicating the existence of a steady-state condition for OH radical formation. The average *p*CBA degradation constant (k_{exp}) with standard deviation , calculated from the measurements taken at the three stages (initial, middle and final) of the disinfection experiment, are presented in Table 1.

2.5. Photocatalytic TiO_2 chemistry

The eight major photochemical TiO_2 reactions are presented in Table 2. The first step involves light inducing the generation of a hole (h^+_{vb})/electron (e^-_{cb}) pair in the valence band (VB) and conduction band (CB), respectively (reaction (I)). This e^-_{cb} (conduction band electron) is available for electron transfer to reducible species that are adsorbed to the TiO_2 surface. The CB electron reduces oxygen to $O_2^-\bullet$ (reaction (II)) and the further reduction of $O_2\bullet^-$ produces H_2O_2 (reaction (III)). Reduction of H_2O_2 by e^-_{cb} can produce the OH radical (reaction (V)). The superoxide can react with H_2O_2 to produce the OH radical (Haber–Weiss reaction, reaction (IV)). The VB hole abstracts electrons from absorbed oxidizable species or reacts with OH^- or H_2O to form the OH radical (reactions (VI) and (VII)). The recombination of OH radicals also produces H_2O_2 (reaction (VIII)). Therefore, the production of H_2O_2 can be ascribed to either a reductive pathway (reaction (III)) or an oxidative pathway (reaction (VIII)).

Table 2
Major TiO_2 photocatalytic reactions

Major reactions	Reaction no.
$TiO_2 \rightarrow^{hv} TiO_2(h^+_{vb} + e^-_{cb})$	(I)
$O_2 + e^-_{cb} \rightarrow O_2\bullet^-$	(II)
$O_2\bullet^- + e^-_{cb} + 2H^+ \rightarrow H_2O_2$	(III)
$O_2\bullet^- + H_2O_2 \rightarrow \bullet OH + OH^- + O_2$	(IV)
$e^-_{cb} + H_2O_2 \rightarrow \bullet OH + OH^-$	(V)
$h^+_{vb} + OH^- \rightarrow \bullet OH$	(VI)
$h^+_{vb} + H_2O \rightarrow H^+ \bullet OH$	(VII)
$2\bullet OH \rightarrow H_2O_2$	(VIII)

2.6. The delayed Chick–Watson model

A disinfection kinetic model is usually needed in order to compare the results obtained from the different experimental conditions. The Chick–Watson Model ($\ln N/N_0 = -kCT$) is simple and easy to apply. However, neither the shoulder nor the tailing off of *E. coli* inactivation can be explained using this model. The delayed Chick–Watson Model, which is listed below, successfully demonstrates the merit of explaining the inactivation kinetics including the shoulder [16].

Delayed Chick–Watson Model:

$$\log\frac{N}{N_0} = \begin{pmatrix} 0 & \text{if } CT \leqslant CT_{lag} = \frac{1}{k}\log\left(\frac{N}{N_0}\right) \\ -k(CT - CT_{lag}) & \text{if } CT \geqslant CT_{lag} = \frac{1}{k}\log\left(\frac{N}{N_0}\right) \end{pmatrix} \quad (3)$$

where N_0 is the initial *E. coli* population (cfu/ml), N the remaining *E. coli* population at time t (cfu/ml), C the OH radical concentration (mg/l), k the inactivation rate constant with ozone (l/(mg.min)), and T the inactivation time (min).

The other sophisticated models such as the series event model, the Hom model and the Rational model can have a better advantage in explaining the TiO_2 inactivation kinetics of *E. coli* [17]. However, since delayed Chick–Watson model allows a CT calculation, and consequently possible to compare the previously fitted CT values, which was calculated by delayed Chick–Watson Model with other disinfectants, such as ozone, free chlorine and chlorine dioxide, this model was considered in this study.

3. Results and discussion

3.1. The importance of the OH radical on E. coli inactivation

Fig. 2 presents the results concerning the effect of oxygen and the presence of OH radical scavenger on *E. coli* inactivation in the photocatalytic TiO_2 system (pH 7.1, 20°C and 1.0 g/l TiO_2). As shown in Fig. 2 and Table 1, these disinfection experiments (No. 1–No. 6), which were repeated three times, show a good reproducibility with 10% standard deviation. These results were obtained under three different dissolved oxygen conditions: air equilibration, O_2 saturation, N_2 saturation. Firstly, two levels of oxic conditions were used, in order to examine the effect of oxygen on TiO_2 disinfection. The oxic conditions employed in experiments No. 2 and

5, and No. 3 and 6 were achieved by mechanical mixing and oxygen gas sparging, respectively. A higher dissolved oxygen concentration (>8 mg/l) was maintained in the latter set of experiments (No. 3 and 6). Secondly, experiments No. 1 and 4 were performed under anoxic conditions which were achieved by nitrogen sparging. Thirdly, experiments (No. 4–6) were performed in the presence of the OH radical scavenger (30 mM Methanol), in order to estimate the effect of the OH radical on *E. coli* inactivation in the TiO_2 disinfection application. Methanol is known to act as a hole scavenger as well as an efficient scavenger of free OH radicals and surface OH radicals [18].

Fig. 2 shows that significant inactivation of *E. coli* occurred only in the presence of dissolved O_2 (No. 2 and 3). The photocatalytic inactivation of *E. coli* was more efficient under O_2 saturation than under air equilibration. In the absence of oxygen, no inactivation of *E. coli* was observed (No. 1 and 4). The corresponding photocatalytic *p*CBA degradation profile is compared with that of *E. coli* inactivation in the inset of Fig. 2. This observation can be explained on the basis of the hypothesis that more available dissolved O_2 molecules scavenge more CB electrons with reducing the chance of charge pair recombination and consequently producing more OH radicals. On the other hand, in the absence of O_2, all photogenerated charge pairs should recombine with no production of OH radicals or ROS since there are no CB electron scavengers [5]. The presence of MeOH significantly inhibited *E. coli* inactivation, with the extent of inactivation being less than 0.5 log in 60 min (No. 5 and 6). It should be noted that there was no difference in the inactivation efficiency between the air-equilibrated and O_2-saturated suspensions in the presence of MeOH. Even in the presence of OH radical scavengers (MeOH), other ROS such as $O_2\bullet^-$ or H_2O_2 can be formed on the TiO_2 surface. This observation implies that the superoxides alone (generated via reaction (II)) play a minor role in the inactivation mechanism of microorganisms. This explanation is consistent with the fact that no *p*CBA degradation took place either in the absence of dissolved O_2 or in the presence of MeOH (i.e., under the condition where the OH radical production is inhibited). It is now well accepted that the main photooxidant in the photocatalytic degradation of recalcitrant organic pollutants is OH radicals [19,20], and that the oxidizing power of other ROS, which are surely generated on TiO_2 surface along with OH radicals is not strong enough to achieve efficient degradation of chemicals. However, it should be noted that ROS ($O_2\bullet^-$, H_2O_2) alone has some reduced inactivating activity for microorganisms.

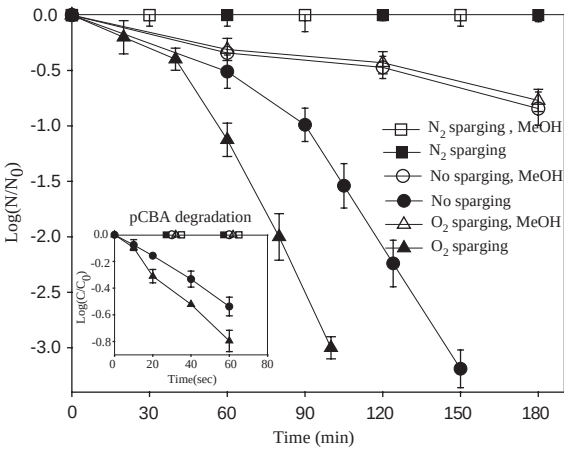

Fig. 2. Effect of oxygen and OH radical scavenger (MeOH = 30 mM) on inactivation of *E. coli* (pH 7.1, TiO_2 1.0 g/l, 20°C, light intensity: 7.9×10^{-6} Einstein/l s). (-■-) No. 1, (-●-) No. 2, (-▲-) No. 3, (-□-) No. 4, (-○-) No. 5, and (-△-) No. 6.

3.2. Effect of TiO_2 concentration

Experiments were performed to investigate the effect of TiO_2 concentration on the photocatalytic inactivation of *E. coli* (Fig. 3(a) and (b)). Fig. 3(a) shows a significant dependence of the *E. coli* inactivation efficiency on the

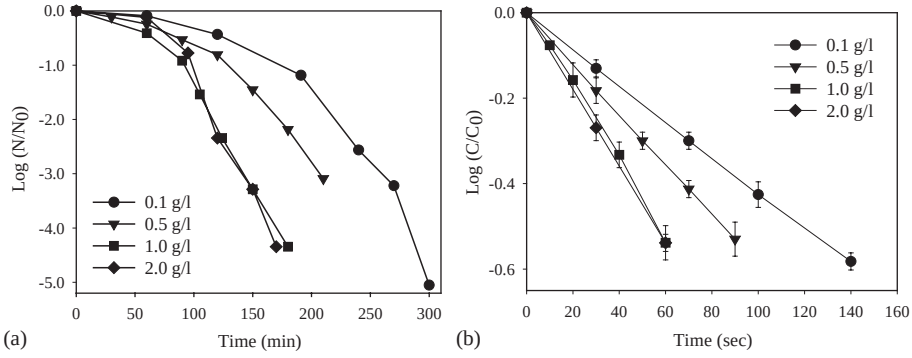

Fig. 3. Effect of TiO_2 concentration on inactivation of *E. coli* (pH 7.1, 20°C, light intensity: 7.9×10^{-6} Einstein/l s). (-●-) No. 7, (-▼-) No. 8, (-■-) No. 2, and (-◆-) No. 9. (a) *E. coli* inactivation; and (b) *p*CBA degradation.

TiO_2 concentration. A similar dependence on the TiO_2 concentration is also observed for *p*CBA degradation as shown in Fig. 3(b). As reported earlier [5,6,8], higher efficiencies of *E. coli* inactivation were obtained at higher TiO_2 concentrations. However the extent of *E. coli* inactivation was not linearly proportional to the TiO_2 concentration, as shown in Fig. 3(a). This means that a higher TiO_2 concentration generates more reactive species responsible for *E. coli* inactivation, but not in a proportional manner. *E. coli* inactivation at a concentration of 1.0 g/l TiO_2 is approximately two times more effective than that at 0.1 g/l TiO_2 concentration. However, increasing the TiO_2 concentration from 1.0 to 2.0 g/l did not enhance the inactivation efficiency of *E. coli*. A similar behavior is observed in Fig. 3(b), which shows that the *p*CBA degradation is almost the same between the TiO_2 concentration of 1.0 and 2.0 g/l. The saturating photoactivity with increasing TiO_2 concentration should be understood in terms of the competition between surface area and light scattering loss. While the higher TiO_2 concentration provides more surface sites, it also decreases the light penetration depth into the suspension through increasing light scattering, which reduces the efficiency of the photocatalytic inactivation of *E. coli* [8,13].

3.3. Effect of light intensity

The log inactivation of *E. coli* and corresponding *p*CBA degradation with irradiation time are presented in Fig. 4(a) and (b), respectively. Fig. 4 shows that more rapid inactivation occurred at higher light intensity, although not in a proportional manner. *E. coli* inactivation with the four lamps (7.9×10^{-6} Einstein/l s) is approximately two times more efficient than that with one lamp (1.5×10^{-6} Einstein/l s). The time required for 2 log *E. coli* inactivation and *p*CBA degradation showed a square-root dependence on light intensity (the inset in Fig. 4, $R^2 = 0.98$). This observation agrees with the results of previous studies [3,6,8].

3.4. Effect of temperature

It is well known that microbial inactivation becomes slower at lower temperatures, when chemical disinfectants such as chlorine and ozone are applied [19]. This is because the metabolism of the microorganism responds more slowly to these chemicals at lower temperatures. The inactivation characteristics of *E. coli* were examined at three different temperatures (6°C, 20°C, 33°C), and compared with the temperature dependence of the *p*CBA degradation. Fig. 5(b) shows that *p*CBA degradation is faster at higher temperature, which implies that OH radical formation is enhanced at higher temperature. The temperature dependence of light intensity of the BLB lamps employed in this study, which can be shown in Table 1 (No. 2, [12,13]), successfully explains the difference of *p*CBA degradation. This means that no significant difference of *p*CBA degradation is expected if the same light intensity had been applied, which is consistent with no temperature dependence of photochemical reaction [21].

However, the inactivation of *E. coli* was observed to be much faster than expected at higher temperature, exceeding the anticipated increase resulting from the formation of OH radicals at higher temperature. As the temperatures increased from 20°C to 33°C, the time required for 0.5 log reduction of *p*CBA concentration and *E. coli* population decreased by 27% and 75%, respectively. This implies that the effect of temperature on *E. coli* inactivation in photocatalytic TiO_2 disinfection is mainly due to the change in microorganism susceptibility.

3.5. Effect of pH

Fig. 5 shows the effect of pH on *E. coli* inactivation, as the pH was changed from 5.6 to 8.1. As shown in Fig. 5(a) and (b), no significant pH effect, either on *E. coli* inactivation or on *p*CBA degradation, was observed at the pH conditions used in this study. The

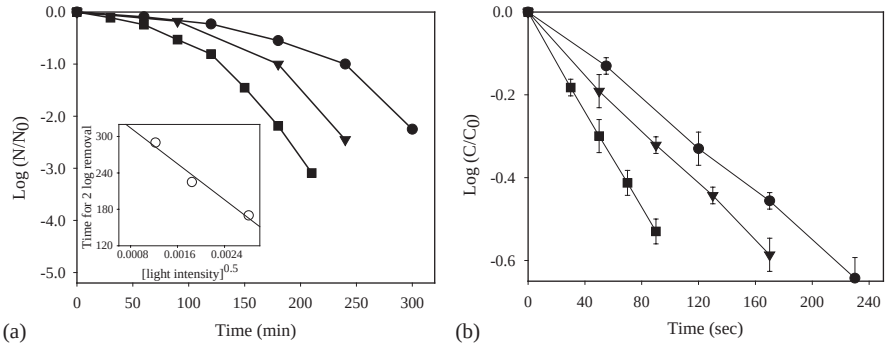

Fig. 4. Effect of light intensity on inactivation of *E. coli* (pH 7.1, TiO_2 0.5 g/l, 20°C). (-●-) No. 10; 1.5×10^{-6} Einstein/l s, (-▼-) No. 11; 3.4×10^{-6} Einstein/l s, and (-■-) No. 8; 7.9×10^{-6} Einstein/l s. (a) *E. coli* inactivation; and (b) *p*CBA degradation.

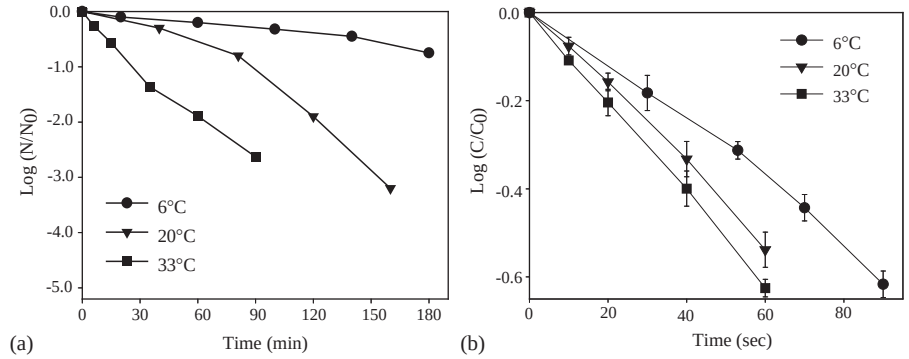

Fig. 5. Effect of temperature on inactivation of *E. coli* (pH 7.1, TiO_2 1.0 g/l, light intensity: 7.9×10^{-6} Einstein/l s). (-●-) No. 12, (-▼-) No. 2, and (-■-) No. 13. (a) *E. coli* inactivation; and (b) *p*CBA degradation.

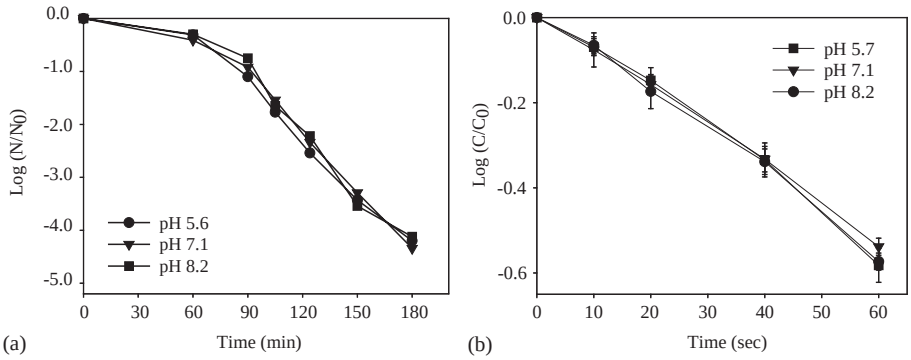

Fig. 6. Effect of pH on inactivation of *E. coli* (TiO_2 1.0 g/l, 20°C, light intensity: 7.9×10^{-6} Einstein/l s). (-●-) No. 14, (-▼-) No. 2, and (-■-) No. 15. (a) *E. coli* inactivation; and (b) *p*CBA degradation.

cell surface charge of *E. coli* has a negative charge character and the TiO_2 particle has a point of zero-charge pH of about 6.3. Thus, it was expected that electrostatic repulsion between the TiO_2 surface and *E. coli* at high pH would be higher due to their both having the same negative charge. However, such a trend was not observed. Although the electrostatic interaction at the TiO_2/water interface could be important in many cases of photocatalytic degradation of charged substrates [22], it is a relatively weak force, which can be dominated by other factors. For example, Kim and Choi [19] recently reported that the rate of photocatalytic degradation of a cationic substrate, tetramethylammonium, was comparable between acidic and basic pH conditions under which the electrostatic interaction is opposite. They proposed that the presence of diffusing OH radicals in the TiO_2 suspension could be responsible for this behavior. The role of electrostatic interaction between the charged cell surface and TiO_2 surface could be insignificant in determining the overall photocatalytic activity. The main surface interaction that affects the photocatalytic inactivation of microorganisms needs to be further investigated.

3.6. OH radical formation vs. E. coli inactivation

The delayed Chick–Watson model was applied to all the results (Figs. 2, 3, 4, 6) obtained at 20°C in order to examine the fitness of model prediction and determine the *CT* value of OH radical for *E. coli* inactivation. The results of these calculations are presented in Fig. 7 (a) and (b). As shown in Fig. 7 (a), the applicability of the delayed Chick–Watson model is excellent with a high R^2 value of 0.91. From Fig. 7 (b), the OH radical *CT* for achieving 2 log *E. coli* inactivation was initially found to be 0.8×10^{-5} mg min/l. However, it should be noted that this value was obtained under the assumption that the OH radical is the only major species responsible for *E. coli* inactivation. This value is overestimated by one-fourth (for 120 min), judging from the result shown in Fig. 2 since one-fourth of *E. coli* inactivation should be ascribed to ROS (not including OH radicals). The above *CT* value (for 2 log inactivation) implies that the OH radical is approximately a thousand or even ten thousand times as effective for *E. coli* inactivation as common disinfectants such as chlorine (1.3×10^{-1} mg min/l), ozone (4.0×10^{-2} mg min/l) and chlorine dioxide (8.0×10^{-2} mg min/l) [23,24].

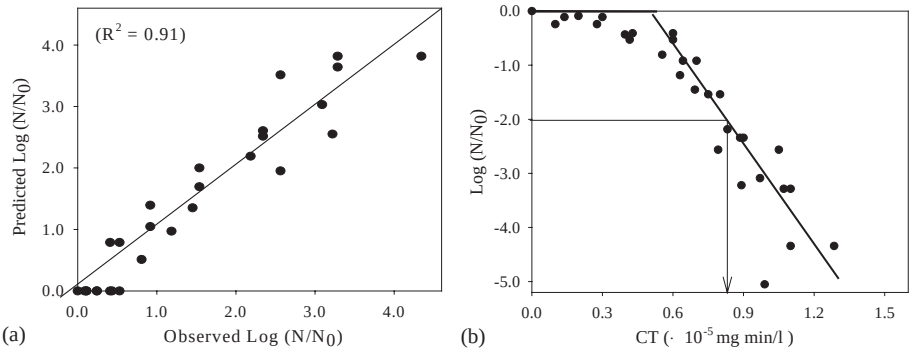

Fig. 7. Determination of *CT* values of OH radical from the delayed Chick–Watson Model prediction at 20°C (Experimental conditions: TiO_2 concentration; 0.1–2.0 g/l, light intensity; 1.5–7.9 × 10^{-6} Einstein/l s, pH; 5.7–8.2). (a) Evaluation of fit; and (b) model prediction.

The correlation between the OH radical concentration and the inactivation of *E. coli* at 20°C was examined among experimental data obtained under different reaction conditions (Figs. 2–4, 6) and shown in Fig. 8. An excellent linear correlation is exhibited between the steady-state concentration of OH radical and the extent of *E. coli* inactivation at the same temperature (R^2 = 0.97 at 20°C). This could be expected based on the good applicability of the delayed Chick–Watson model, as demonstrated in Fig. 7. The good linear relationship strongly supports the hypothesis that the OH radical produced in the TiO_2 photocatalytic reaction is the major species responsible for *E. coli* inactivation. As explained, because inactivation of *E. coli* affected not only generated OH radical but also the change of microorganism susceptibility at different temperature, the results of other temperatures, 6°C and 33°C were not considered.

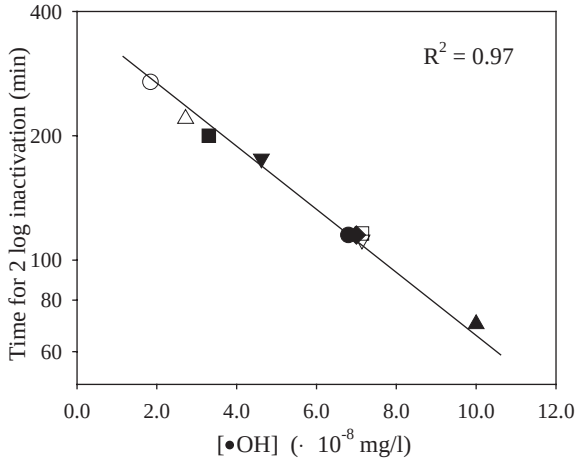

Fig. 8. Linear relationship between the OH radical concentration and the extent of *E. coli* inactivation (Experimental conditions : No. 2 : TiO_2 1 g/l, light intensity 7.9 × 10^{-6} Einstein/l s, pH 7.1; (▲) No. 3 : TiO_2 1 g/l, light intensity 7.9 × 10^{-6} Einstein/l s, pH 7.1, O_2 sparging; (■) No. 7 : TiO_2 0.1 g/l, light intensity 7.9 × 10^{-6} Einstein/l s, pH 7.1; (▼) No. 8 : TiO_2 0.5 g/l, light intensity 7.9 × 10^{-6} Einstein/l s, pH 7.1; (♦) No. 9 : TiO_2 2 g/l, light intensity 7.9 × 10^{-6} Einstein/l s, pH 7.1; (○) No. 10 : TiO_2 0.5 g/l, light intensity 1.5 × 10^{-6} Einstein/l s, pH 7.1; (—) No. 11 : TiO_2 0.5 g/l, light intensity 3.4 × 10^{-6} Einstein/l s, pH 7.1; (□) No. 14 : TiO_2 1 g/l, light intensity 7.9 × 10^{-6} Einstein/l s, pH 5.7; and (□) No. 15 : TiO_2 1 g/l, light intensity 7.9 × 10^{-6} Einstein/l s, pH 8.2).

3.7. Inactivation curve of E. coli

In many previous studies [1,2,5,8,25], the exact *E. coli* inactivation curve was not clear because the time scale of reported inactivation studies was limited to one log inactivation. As shown in Figs. 2–6, all of the *E. coli* inactivation curves were found to be of the shoulder type which exhibited a lag phase during the early inactivation period, except at high temperature (No. 13). Therefore, when the photocatalytic disinfection is applied to the water treatment system, where multiple log inactivation is required, the shoulder-type kinetics of *E. coli* inactivation needs to be considered.

4. Conclusion

This study demonstrates an excellent linear correlation between the amount of OH radical and the extent of *E. coli* inactivation in TiO_2 photocatalytic disinfection at constant reaction temperature, which indicates that the OH radical is the primary species responsible for *E. coli* inactivation. The OH radical *CT* for achieving 2 log *E. coli* inactivation was initially found to be 0.8 × 10^{-5} mg min/l, as predicted by the delayed Chick–Watson model. This *CT* value implies that the OH radical is approximately a thousand to ten thousand times as effective for *E. coli* inactivation as other

chemical disinfectants such as chlorine, ozone and chlorine dioxide.

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