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Influences of humic acids and photoreactivation on the disinfection of *Escherichia coli* by a high-power pulsed UV irradiation

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Abstract−The characteristics of inactivation and photoreactivation of *Escherichia coli* by a high-power pulsed ultraviolet (PUV) irradiation and the influence of the humic acids on disinfection performance were investigated. The pulsed power source was operated at 2,400 V, with 200 J of energy being stored in a 100-μF capacitor. This energy dissipated in the xenon-filled flashlamp within 150 μs, generating a megawatt-level peak power per pulse. The light source was operated at 12 pulses per second (12 Hz). More than a 6-log reduction was achieved via 5 sec of irradiation at a distance of 20 cm, which corresponds approximately to a UV dose of 23 mJ/cm^2. The inactivation efficiency decreased with increasing concentrations of humic acids (HA). At the distance of 20 cm, 10 ppm HA reduced the inactivation efficiency to 50%. UV absorption by HA and, in part, the growth-promoting effect of humic acids were implicated in reductions of inactivation performance in the presence of HA. After a disinfection procedure with PUV, the possibility of photoreactivation was greatly reduced and an additional reduction of approximately 1-log was also achieved during 8 h under visible light after inactivation with a UV dosage of 9.0 mJ/cm^2 (3 sec at 30 cm). The extent of additional inactivation under visible light was reduced with increasing concentrations of humic acids in water.

Key words: Disinfection, Pulsed-UV Irradiation, Xenon Flashlamp, Photoreactivation, Humic Acids

INTRODUCTION

Ultraviolet (UV) irradiation has become an attractive option for the disinfection of water, air, food surfaces, and pharmaceutical packaging, due principally to its ability to inactivate microorganisms with less concern about byproduct formation and overdose, as well as the simplicity of maintenance associated with UV protocols [1-4]. The UV-induced inactivation of microorganisms is attributable to the DNA absorption of UV light, which causes the formation of thymine dimers that disrupt the normal process of DNA replication [5-7].

Two conventional sources of UV light are low-pressure (LP) and medium-pressure (MP) mercury lamps. Low-pressure mercury lamps employ 100-200 Pa pressure and emit a monochromatic UV line at 253.7 nm. Medium-pressure lamps provide increased power by using pressures of hundreds of kPa, and emit several polychromatic lines between 200 and 300 nm. Both types of lamp utilize continuous UV light emission. UV disinfection is considered to be inefficient when the water is highly turbid or contains UV-absorbing materials [8-10]. Another concern is that certain microorganisms can be reactivated, particularly in cases in which disinfected water is returned back to visible light. This phenomenon has been attributed to a light-induced DNA repair mechanism [11-14].

An alternative to the use of continuously emitting mercury lamps as a source of intense UV radiation is the use of flashlamps capable of emitting UV light in a pulsed fashion. Pulsed UV (PUV) sources are a relatively new lamp technology, which was first developed to increase irradiation power and the depth of UV transmittance, as well as to employ a broader band of germicidal UV lines for disinfection. Flash-type UV lamps, such as pulsed xenon or pulsed krypton lamps, intermittently emit high energy and a wide range of UV lines. It is known, particularly with xenon, that when a high-energy pulse is applied for a duration of milliseconds or less, the xenon ions are excited, which results in the emission of a broad and strong polychromatic spectrum of ultraviolet and visible as well as infrared light, including effective germicidal UV lines [15-17].

Although PUV technology has been previously used in food and pharmaceutical packaging for sterilization, the commercial application of this technology to water treatment is, thus far, somewhat limited. Pulsed light of UV range generated from a xenon or krypton flash lamp is known to be effective in the inactivation of *E. coli* and bacteriophages [13,14,18,19]. Recently, it has been reported that PUV irradiation is highly efficient in the inactivation of *Cryptosporidium* oocysts [19,20]. PUV has been previously shown to have advantages over continuous UV light sources in situations where rapid disinfection is required [15,16,19].

In this study, we investigated the inactivation efficiency of a recently developed pulsed UV system for possible use in water disinfection. The effects of irradiation distance and the duration of UV exposure on *E. coli* inactivation were assessed.

Natural organic matters (NOMs) such as humic acids (HA) in water are the principal constituents generating disinfection byproducts upon reaction with chlorine [22-25]. Humic acids are also known to absorb UV, and thus the UV transmittance decreases with increasing concentrations of these compounds in water [3,26]. Thus, the extent of humic acid interference on the inactivation of *E. coli* was assessed in this study.

One of the problems associated with UV disinfection involves

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the mechanisms of microbial repair. Many microorganisms, including *E. coli*, feature enzyme systems that repair the damages caused by UV light. In particular, photoreactivation (or photorepair) involves the repair of pyrimidine dimers in the presence of near-UV or visible light at wavelengths 310-490 nm [11,13]. Repair mechanisms permit cells to regain their viability after UV disinfection, and thus an increased UV dose is required to achieve a given degree of inactivation. As there have been relatively few reports addressing reactivation after pulsed UV irradiation, we attempted to evaluate the extent of reactivation of *E. coli*, and have included a discussion of the difference in the reactivation characteristics between pulsed-UV and LP or MP UV irradiation.

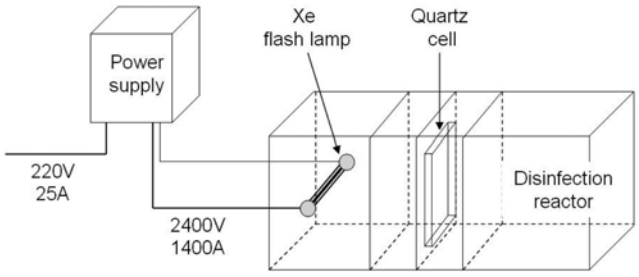

Fig. 1. A schematic of the pulsed UV irradiation system used in this study.

MATERIALS AND METHODS

1. *E. coli* Cells

Escherichia coli (ATCC 15597) was inoculated into a flask containing tryptic soy broth (TSB) and incubated for 24 h in a shaking incubator at 37 °C and 150 rpm. The cells were diluted to 10^5-10^7 cfu/mL prior to the application of UV radiation, which was administered after the cell population exceeded 10^8 cfu/mL. After the inactivation experiment, the samples were diluted appropriately and plated on tryptic soy agar, then incubated for 24 h at 37 °C, and the number of survived colonies of *E. coli* were counted and recorded.

2. Pulsed-UV System

The pulsed UV system consisted of a disinfection reactor equipped with a xenon-filled flash lamp and a pulsed UV light source (PS2000, Green EnTech, Inc., Seoul, Korea), as is shown in Fig. 1. The pulsed UV light source is a power supply which transfers stored electric energy using a power compression technology to the Xe flash lamp for a short duration at a high peak power. The pulsed power source was operated at 2,400 V, with 200 J of energy being stored in a 100-μF capacitor. This stored energy is dissipated in the xenon-filled flash lamp within 150 μsec, thus generating a peak power per pulse of approximately 2 MW [27,28]. This light source was operated at 12 pulses per second (12 Hz) in this study.

This system generates a high-intensity diverging beam of polychromatic pulsed light, and the spectrum of the generated radiation encompasses the UV, visible, and infrared regions, in a range from 200 to 900 nm [21,27]. Fig. 2 shows the spectra of pulsed-UV light generated from the Xe flash lamp in a range of 220-400 nm, and its variations at different operating voltages (Ocean Optics Spectrometer USB4000, Dunedin, FL, USA). The magnitude of irradiance was substantially increased at 2,400 V, compared to the values observed at lower voltages. This is the inherent property of flashlamps employing xenon or krypton. By delivering a high power electrical pulse in millisecond burst, xenon and krypton emits such an intense and wide spectrum covering UV, visible and IR range, especially with enhanced UV range. To induce such emission with a broad and high irradiance, a certain threshold value of voltage (or power) should be applied, and below which the emitting spectrum is monotonous at low irradiance as shown in Fig. 2. The threshold value observed in the system used in this study was about 2,200 V. Similar patterns of emitting spectrum have been mentioned by others [15-17]. The UV light irradiance measured in the range of wavelength 200-400 nm was 4.64, 3.00, 2.23 mW/cm^2 at distances of 20, 30, 40 cm, respectively, as is shown in Table 1 [21].

The disinfection reactor was a stainless steel chamber (62×32×19 cm) with a working volume of 18 L and a Xe flash lamp (overall length 190 mm, arc length 150 mm, diameter 7 mm) submerged horizontally in the water. The test sample was loaded into a 300-mL quartz cell (4×3×28 cm). The quartz cell was constructed of acrylic resin, but the front face of the cell was formulated of 5 mm-

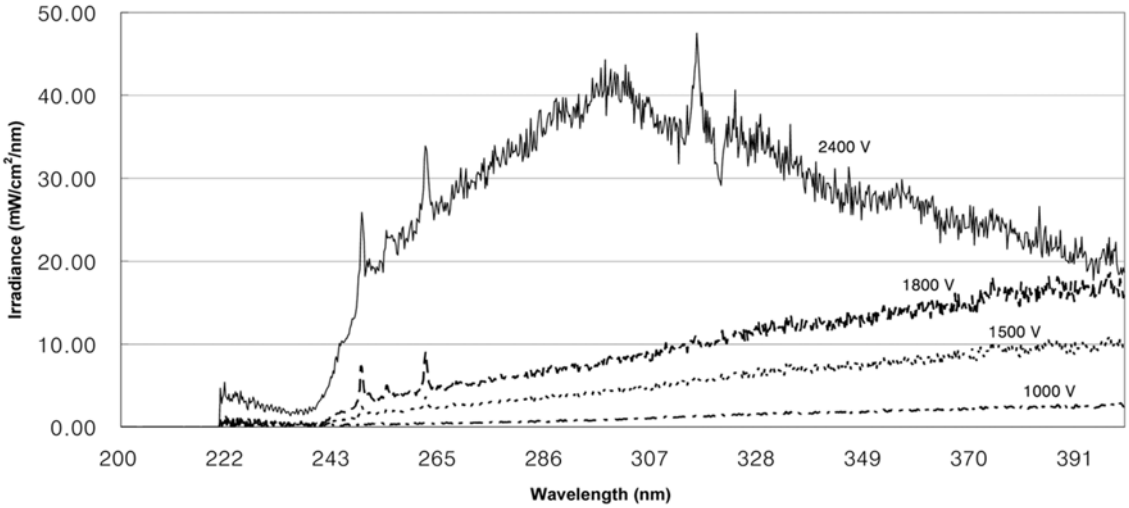

Fig. 2. Spectra and irradiance produced by the Xe flashlamp in the 220-400 nm range.

Table 1. UV intensity and dose at each location and duration used in the experiments

Distance (cm)		20	30	40
UV irradiance (mW/cm^2)[a]		4.6	3.0	2.2
UV dose (mJ/cm^2)	3 sec	13.8	9.0	6.6
	5 sec	23.0	15.0	11.0

[a]from Lee *et al*. (2008)

thick quartz in order to minimize the absorption and diffraction of UV light during the irradiation experiments. The reactor chamber has frames to hold the quartz cell at different distances from the lamp. UV disinfection was conducted in static mode without water circulating.

3. Humic Acids

The humic acids used in this study were a mixture called Suwannee River Humic Acid Standard, which was supplied by the International Humic Substances Society (IHSS), GA, USA. The mixture of humic acids was dissolved in distilled deionized water at concentrations of 0 to 20 ppm. *E. coli* cells were spiked and then pulsed UV disinfection and photoreactivation experiments were conducted.

4. Inactivation

The disinfection reactor was filled with 18 L of tap water and the quartz cell containing a 300 mL water sample with a known concentration of *E. coli* cells. The light source was operated at 12 pulses per second, and the distance from the UV lamp to the quartz cell was varied, and set at 20, 30, and 40 cm. The UV light irradiance measured in a wavelength range of 200-400 nm was 4.64, 3.00, 2.23 mW/cm^2 at distances of 20, 30, and 40 cm, respectively [21,28]. The durations of pulsed irradiation utilized in this study were 3 and 5 sec. The initial temperature of the water was 20 °C, and we noted no discernible increases in water temperature during the experiments.

5. Photoreactivation After Pulsed UV Irradiation

A water sample containing *E. coli* cells at concentrations of 10^7 cfu/mL or more was irradiated for 3 sec with pulsed UV light at a distance of 30 cm, after which the irradiated sample was divided in two. One fraction was stored in a dark chamber, and the other under visible light. A 40 W fluorescent lamp was horizontally placed 20 cm above the liquid surface in a petri dish. The cell population was monitored as a function of incubation time for 10 hours at 20 °C via the plate count method on tryptic soy agar.

RESULTS AND DISCUSSION

1. Inactivation Characteristics

The disinfection efficiency measured at different distances and irradiation times for the inactivation of *E. coli* was assessed by using the pulsed UV irradiation system. The light source was operated at 12 pulses per second (12 Hz). The distance between the UV lamp and the quartz cell was set at 20, 30, and 40 cm. The durations of pulsed irradiation utilized in this study were 3 and 5 sec. An initial cell concentration of at least 10^7 cfu/mL was used for all experiments.

Fig. 3 shows that a 6.4-log reduction was achieved after 5 sec of irradiation at a distance of 20 cm. As the distance between the sample and the lamp was increased, the inactivation efficiency decreased

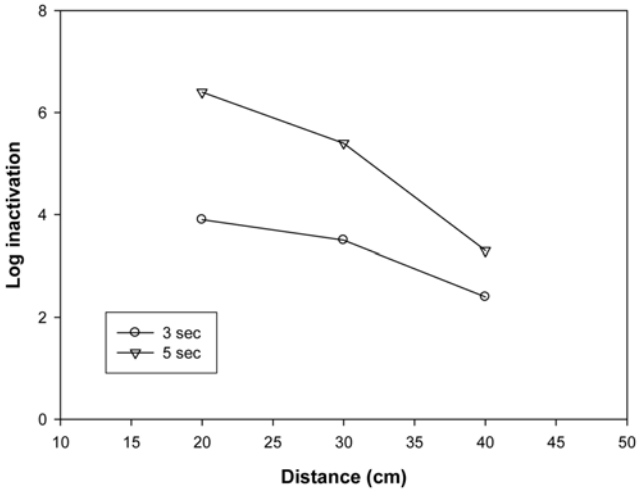

Fig. 3. Inactivation at different distances and irradiation times.

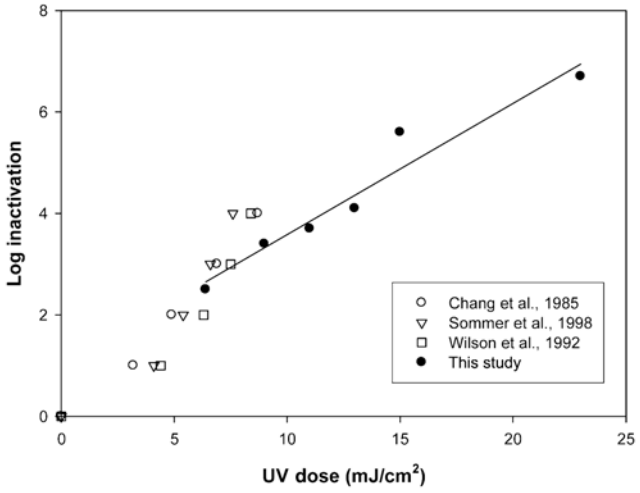

Fig. 4. Inactivation levels at different UV doses from this study using pulsed UV irradiation and other studies using LP and MP UV irradiation.

to a 5.5-log reduction and a 3.5-log reduction at 30 cm and 40 cm, respectively. With 3 sec of irradiation, the achieved reductions at the distances of 20, 30, and 40 cm were 4.0-log, 3.7-log, and 2.4-log, respectively. The inactivation efficiency determined at 3 sec of irradiation was approximately 65-70% that achieved at 5 sec.

The achieved values of log-inactivation at different UV doses are plotted in Fig. 4. Here, the values of UV dosage were based on the UV light irradiance (Table 1) measured with this system previously in a UV wavelength range of 200-400 nm [21]. Dosages of 5 mJ/cm^2 and 10 mJ/cm^2 were estimated to result in the inactivation of approximately 2-2.5 and 3.5-4-log, respectively, and this performance was similar to that reported in other studies in which LP and MP mercury lamps were utilized, as is shown in Fig. 4 together [3,17,30]. Thus, if we assume that a UV dose of approximately 10 mJ/cm^2 is required to achieve a 4-log reduction in *E. coli*, whereas conventional LP or MP UV lamps with 0.2 mW/cm^2 irradiance would require approximately 50 sec to achieve a 4-log inactivation; the pulsed UV system used in this study requires 3 sec at a distance of

20 cm or 5 sec at a distance of 35 cm.

On the basis of the dose values shown in Fig. 4, we noted no significant improvements in terms of the dose required to achieve a given level of inactivation, when our data was compared to several published studies in which continuously-irradiating LP or MP mercury lamps were used. Some studies have reported improved disinfection efficiency with pulsed UV flashlamps as compared to continuous-wave LP or MP lamps, owing to the wider range of the wavelength bands of UV and other light that are seen with the UV-C generated from pulsed xenon lamps [16]. However, the required level of inactivation performance was achieved successfully within a few seconds of irradiation with the pulsed xenon UV irradiation system used in this study. Therefore, it was confirmed that an approach involving short pulse width with an intense UV dose provides practical advantages over approaches involving continuous UV sources in the treatment of water or wastewater, and in cases in which rapid disinfection is required.

2. The Influence of Humic Acids

Fig. 5 shows that the inactivation efficiency decreased with increasing humic acid concentrations and increasing distance from the pulsed UV lamp. At a distance of 20 cm, the presence of 10 ppm HA reduced the inactivation efficiency to 50% of the performance in the absence of HA, and only a 15-20% inactivation was achieved in water containing 20 ppm HA. In the presence of humic acids, the inactivation efficiency at 40 cm was approximately 50% of that at 20 cm, thereby implying that the degradation of inactivation performance became larger with the presence of HA in the water.

The principal cause of the reduced inactivation performance in the presence of HA appears to be the UV absorption characteristics of HA. As is shown in Fig. 6(a), the IHSS humic acid mixture used in this study actively absorbed light in certain ranges, particularly in the UV-C range. The absorbance increased with increasing HA contents. The wavelength that was most effectively absorbed in the germicidal range (200-280 nm) was 236 nm, and a substantial fraction of the 254 nm light, which is the wavelength most readily absorbed by DNA, was also absorbed (Fig. 6(b)). The reduction of UV-C transmittance must have caused the degradation of *E. coli* inactivation. No noticeable absorption in visible light range was observed, although the water with a high HA content evidenced a slightly dark-brownish color value.

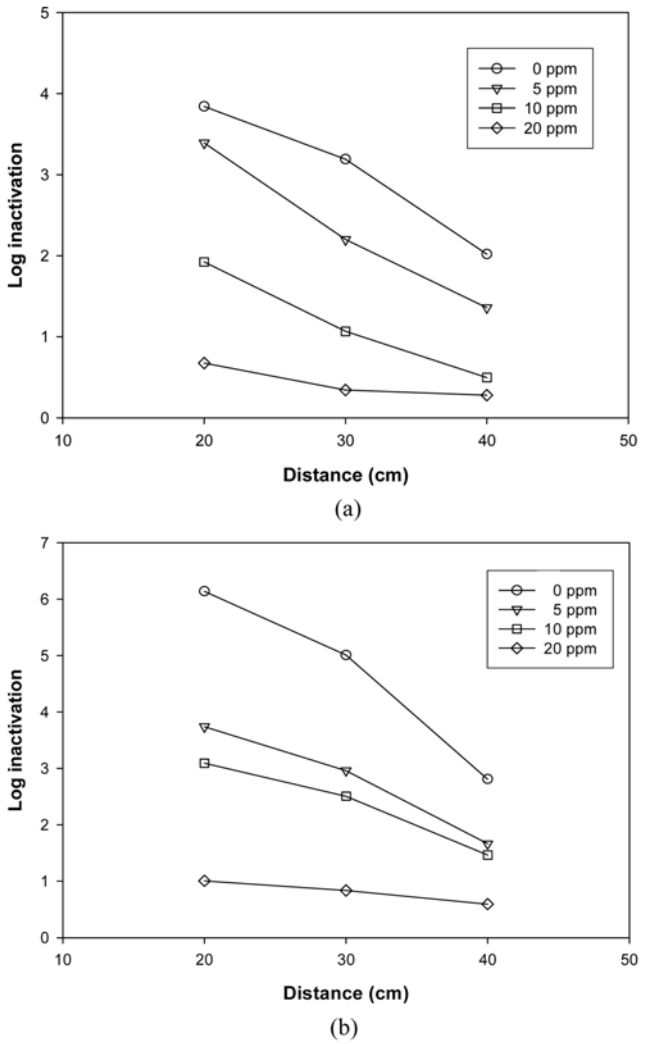

Fig. 5. Effect of humic acids on *E. coli* inactivation by pulsed UV irradiation. (a) 3 sec and (b) 5 sec of irradiation time.

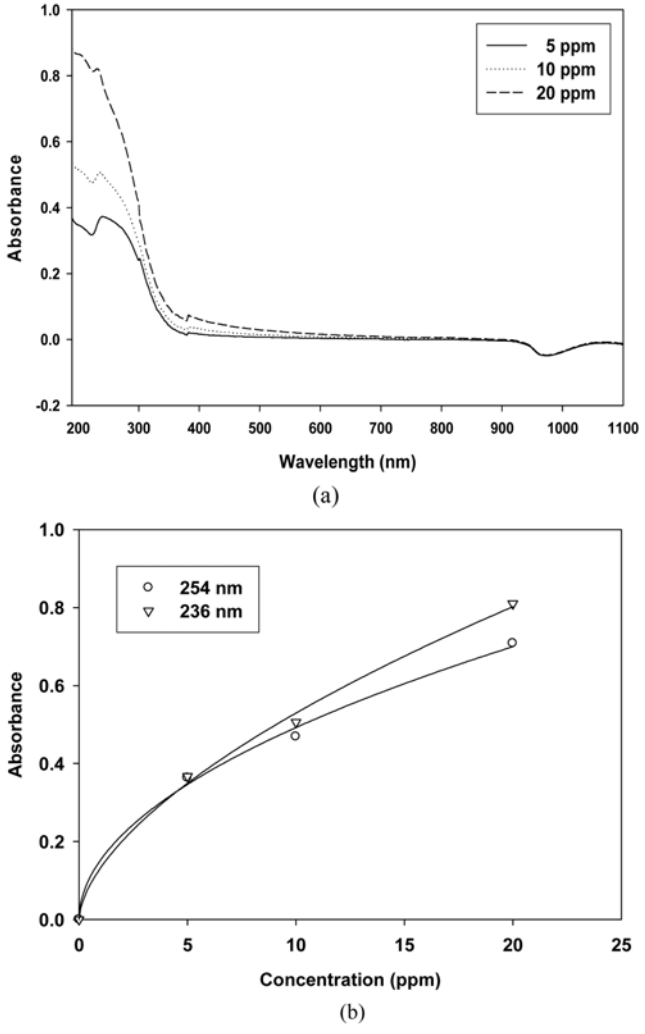

Fig. 6. (a) The absorbance spectrum of the humic acid mixture at different concentrations and (b) the extent of UV absorption by humic acids at 254 nm and 236 nm.

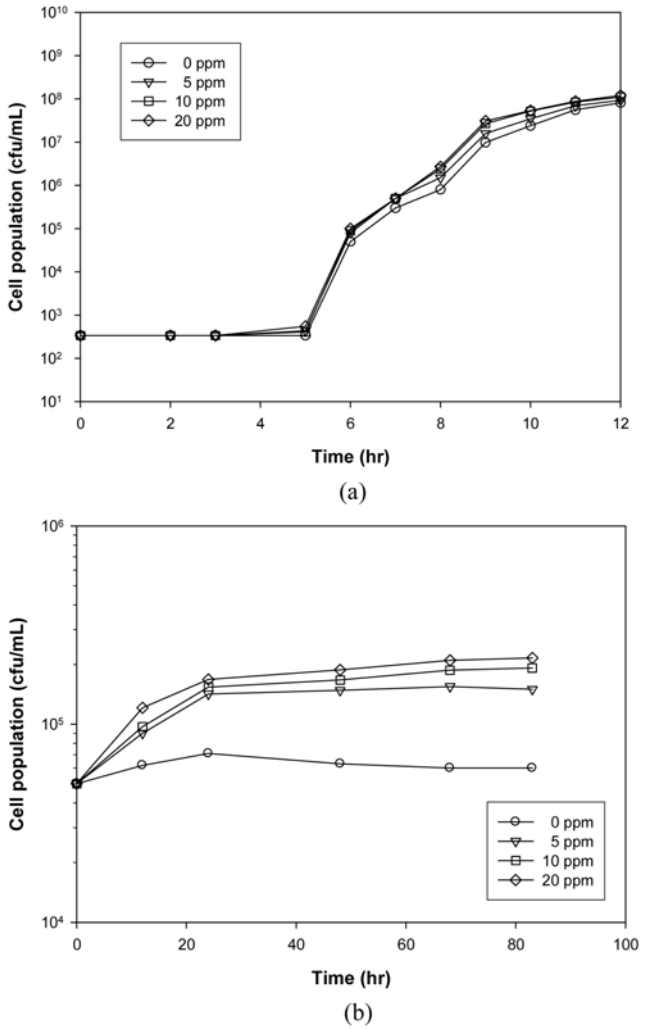

Fig. 7. The growth curves of *E. coli* with and without humic acids in (a) TSB growth medium and (b) distilled water.

Another possible reason for the reduction of UV inactivation performance in the presence of HA was the microorganism growth-promoting effect of HA, and this was also considered in this study. Here, the growth of *E. coli* with and without HA was compared to determine whether or not humic acids actually promote the growth of *E. coli*. Cells were inoculated in regular culture medium (TSB medium) and in fresh water, both containing different concentrations of HA, and the changes in population were monitored. Fig. 7(a) demonstrates that an HA content of 0-20 ppm in growth medium did not induce a significant difference in the overall growth rate, although a slightly higher growth rate during the exponential stage was observed at higher concentrations of HA. However, the growth rate observed in the presence of HA in clean water was significantly increased as compared to what was observed in the absence of HA (Fig. 7(b)). The cell population in water was stably maintained, evidencing no significant growth in the absence of HA, whereas the population was almost doubled after 20 h in the presence of HA.

These results showed that humic acids manifestly promoted the growth of *E. coli*, which may have led to the observed reduction in the disinfection efficiency of UV irradiation protocols. It has been well established that humic acids or HA-rich soils (such as humus)

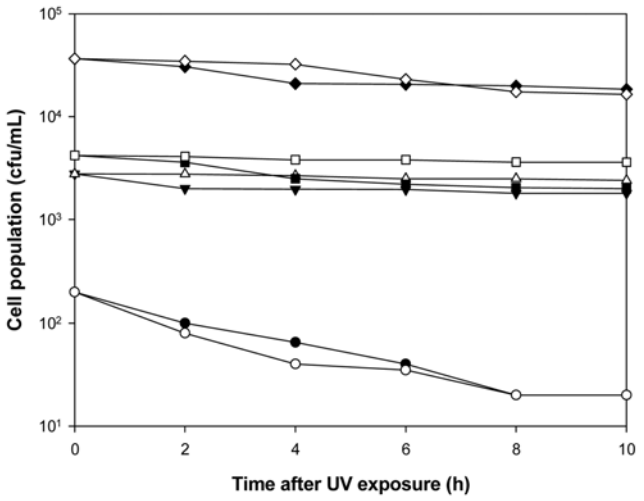

Fig. 8. The reactivation of *E. coli* in the presence of humic acids after pulsed UV irradiation stopped. Initial cell concentration before UV exposure was N_0=~10^7 cfu/mL. UV irradiation: 3 sec at distance 30 cm (UV dose: 9 mJ/cm^2). HA concentration: ○ and ●, 0 ppm; △ and ▲, 5 ppm; □ and ■, 10 ppm; ◇ and ◆, 20 ppm. Open symbols: light condition. Solid symbols: dark condition.

can be used to activate plant-related microbe communities in soil and to accelerate the growth of a variety of plants [31,32]. This growth-promotion effect was apparent in fresh water, but was not so obvious in growth medium. This is probably due to the fact that TSB growth medium is a nutrient-rich environment, in which HA is not the limiting component for cell growth and proliferation. Here, the relative extent of contribution between two factors of HA to the loss of inactivation performance, UV absorption effect and growth-promotion effect, remains unclear; thus, further study will be required.

3. Photoreactivation After PUV Irradiation

Fig. 8 shows the changes in the population of surviving cells during the reactivation process occurring subsequent to pulsed UV irradiation. The initial cell concentration (N_0) was greater than 10^7 cfu/mL. Pulsed UV irradiation was administered for 3 sec at a distance of 30 cm (*ca.* 9.0 mJ/cm^2). In the water containing no HA, *E. coli* was inactivated to ~10^2 cfu/mL (○ and ● at time zero). After that inactivation, UV irradiation was stopped and the cells were divided into two portions and stored separately under visible light or in darkness. It can be seen in Fig. 8 that, during exposure to visible light, the cell population (○) decreased further as time passed, and no signs of reactivation were observed for 10 h. The final values of the stabilized cell population at 10 h are summarized in Table 2. An additional reduction of approximately 1-log (90%) was also achieved over a period of 8 h. This result is in stark contrast to the results obtained with MP or LP UV lamps, as discussed earlier [14,33]. The surviving population under visible light (○) was slightly different from the one stored in the dark (●), which strongly implied that photoreactivation did not occur to any significant extent.

When HA was present in the water, the extent of additional inactivation during the exposure to light was attenuated, evidencing only a slight reduction in population over a period of 10 hours. However, we noted no signs of photoreactivation, or of an increase in cell population, even with an HA concentration as high as 20 ppm

Table 2. The change of cell population after pulsed UV irradiation stopped. Initial cell concentration before UV exposure was N_0=~10^7 cfu/mL. UV irradiation: 3 sec at distance 30 cm

HA (ppm)	Storing condition	Cell population		
		t=0 h	t=10 h	
		cfu/mL	cfu/mL	Removal %
0	Light	200	20	90.0
	Dark	200	19	90.5
5	Light	2780	2400	13.7
	Dark	2780	1800	35.3
10	Light	4200	3600	14.3
	Dark	4200	1998	52.4
20	Light	36500	16500	54.8
	Dark	36500	18500	49.3

(◇ and ◆). It appears that strong energy pulses in a wide range of irradiation wavelengths caused cellular damage which persisted longer, or perhaps was irreversible, and thus the results of inactivation were noted even after several generations of DNA duplication.

The reasons for the reduced extent of photoreactivation and additional inactivation under visible light subsequent to treatment with pulsed UV irradiation remain to be clearly elucidated. Coliforms and heterotrophic bacteria generally evidence survival rates several orders higher under light conditions as compared to dark conditions, and their survival curve is consistent with saturation-type behavior with regard to exposure time [30,33]. According to Tosa and Hirata [14], an approximate 3-log inactivation of an *E. coli* strain was achieved by using a UV dose of 9.9 mJ/cm^2, but the cells reactivated and the cellular population increased by 2 orders during 100 min under 0.023 mW/cm^2 of visible light intensity.

It has been reported that the application of high-intensity UV over a short period is more effective for disinfection than when the same dose is applied over a longer exposure period [28]. Additionally, it has been speculated that a wide range of wavelength bands, including UV and wavelengths other than UV-C generated from pulsed xenon lamps, may cause additional damage to DNA duplication and repair systems [16,19], or other unknown enzyme systems of the microbes; this may explain the suppression of normal photoreactivation.

CONCLUSIONS

Pulsed UV irradiation with the xenon flashlamp used in the present study would yield some benefits when applied to highly turbid water or wastewater, and an approach coupling short pulse width with high UV dosage may have some practical advantages over continuous UV sources, especially in cases in which rapid disinfection is required. The required level of inactivation performance was achieved successfully within a few seconds of irradiation, although we noted no significant improvement in terms of the dose required to achieve a given level of inactivation when the results were compared with those produced by continuously-irradiating LP or MP mercury lamps.

The inactivation efficiency decreased with increasing concentrations of humic acids and increasing distance from the pulsed UV source. UV absorption by HA may constitute the principal cause of the reduced inactivation performance observed in the presence of HA. The wavelengths that were absorbed most profoundly in the germicidal range (200-280 nm) were 236 nm and a substantial fraction of 254 nm light; thus, the reduction of UV-C transmittance must have induced the observed degradation of *E. coli* inactivation. Another factor influencing the degradation of inactivation efficiency may be the microbial growth-promotion effects of humic acids. Humic acids clearly promoted the growth of *E. coli*, which may have resulted, at least in part, in the UV-induced reduction of disinfection efficiency.

The possibility of photoreactivation using pulsed UV irradiation was significantly reduced, and even an additional inactivation of *E. coli* was achieved during exposure to visible light. Such an extent of additional inactivation was reduced with increasing concentrations of humic acids in water. It has been speculated that the wide range of wavelength bands, including UV-C, generated by pulsed xenon lamp irradiation induces more lethal or irreversible damage to microbial repair systems, thus exerting suppressive effects on the photoreactivation process.

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