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Rapid Disinfection by Peracetic Acid Combined with UV Irradiation

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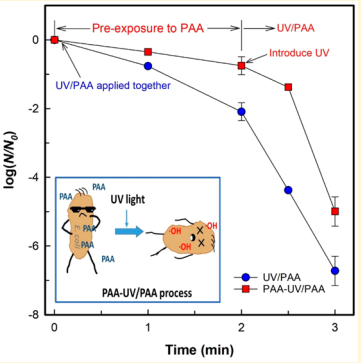
Supporting Information

ABSTRACT: This study proposes a novel disinfection process by sequential application of peracetic acid (PAA) and ultraviolet light (UV), on the basis of elucidation of disinfection mechanisms under UV/PAA. Results show that hydroxyl radicals, generated by UV-activated PAA, contribute to the enhanced inactivation of *Escherichia coli* under UV/PAA compared to PAA alone or UV alone. Furthermore, the location of hydroxyl radical generation is a critical factor. Unlike UV/H_2O_2, which generates hydroxyl radicals mainly in the bulk solution, the hydroxyl radicals under UV/PAA are produced close to or inside *E. coli* cells, due to PAA diffusion. Therefore, hydroxyl radicals exert significantly stronger disinfection power under UV/PAA than under UV/H_2O_2 conditions. Pre-exposing *E. coli* to PAA in the dark followed by application of UV (i.e., a PAA-UV/PAA process) promotes diffusion of PAA to the cells and achieves excellent disinfection efficiency while saving more than half of the energy cost associated with UV compared to simultaneous application of UV and PAA. The effectiveness of this new disinfection strategy has been demonstrated not only in lab water but also in wastewater matrices.



INTRODUCTION

In recent years, peracetic acid (PAA) has been proposed as an alternative disinfectant for replacing chlorine-based oxidants in wastewater treatment.[1−6] The main drivers of using PAA in wastewater treatment include the following: (1) PAA does not generate carcinogenic and mutagenic disinfection byproducts (DBPs),[6,7] which, therefore, greatly benefits the safety and reuse of the treated water. (2) PAA can effectively penetrate and inactivate biofilms.[8] (3) The use of PAA has been shown to be cost effective and can be easily installed in existing wastewater treatment facilities. However, the major drawback of PAA disinfection is that a high PAA dose may increase the organic content in water due to acetic acid (component in PAA stock solution) and thus raises the microbial regrowth potential.[6]

Ultraviolet (UV) disinfection has been well documented due to the effectiveness of inactivation of waterborne pathogens. The inactivation by UV, typically around 260 nm is based on the damage caused to the nucleic acids (DNA/RNA) of the cell or virus.[9] However, UV-resistant organisms, such as viruses, specifically Adenoviruses, and bacterial spores tend to make UV disinfection an energy-intensive technology due to the high UV dose needed to be applied.[10]

Combining UV with PAA is not simply combing two disinfection processes but also generating free radicals, mainly the hydroxyl radical ($^{\bullet}$OH) and acyloxyl radicals,[11] in which $^{\bullet}$OH has been reported to be a powerful disinfectant.[12−14] Several studies have observed the enhancement of inactivation of pathogens by UV/PAA in comparison with UV or PAA alone.[15−18] Koivunen and Heinonen-Tanski observed increased disinfection efficiency of *Escherichia coli*, *Enterococcus faecalis*, *Salmonella enteritidis*, and coliphage MS2 by using combined UV/PAA. They concluded there were synergistic benefits using UV/PAA for all three bacteria except coliphage MS2.[5] Caretti and Lubello conducted a pilot-scale test using secondary effluent to investigate the enhancement of disinfection efficiency by adding PAA upstream and downstream of the UV device. They observed a much higher disinfection efficiency when PAA was applied upstream instead of downstream which was explained by the formation of free radicals due to the photolysis of PAA.[15] By knowing the enhancement of inactivation by UV/PAA, it is important to obtain in-depth knowledge on the mechanisms of UV/PAA disinfection to help the development and design of this advanced technology for effective field applications.

In this work, *E. coli* was chosen to study the mechanisms of inactivation enhancement by UV/PAA. The inactivation of *E. coli* by UV alone and PAA alone was investigated separately and compared with that by UV/PAA. To better understand the role

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of free radicals produced during PAA photolysis, radical quenching experiments were designed, and a disinfection kinetic model was applied. Furthermore, PAA and UV were applied sequentially by controlling the pre-exposure of PAA (and radical scavenger) to better understand the role of free radicals. Specifically, efforts were made to elucidate the synergistic effects between UV, PAA, and hydroxyl radical species in the inactivation of *E. coli*. To the best of our knowledge, this study is among the first to investigate the inactivation mechanism of *E. coli* by sequential application of PAA and UV.

MATERIALS AND METHODS

Materials. Peracetic acid (~39% PAA, ≤ 6% H_2O_2, and ~45% acetic acid by weight) was obtained from Sigma-Aldrich (St. Louis, MO, USA). Sources of other chemicals and reagents are provided in SI Text S1. The concentrations of PAA solution were determined using the iodometric titration method and the *N,N*-diethyl-*p*-phenylenediamine (DPD) titration method,[19] as detailed in SI Text S2. The *E. coli* strain used in this study was obtained from ATCC (ATCC 15597). This strain is commonly used as a host for bacteriophage MS2 and an indicator for pathogenic bacteria. Culture preparation is detailed in SI Text S3. All working solutions (phosphate buffer, PAA, H_2O_2, $Na_2S_2O_3$) were prepared by dissolving/diluting the purchased chemicals into/with sterilized Milli-Q water. All glassware and materials (i.e., reactor) were sterilized before use.

Experimental Setup. The UV-based disinfection experiments were conducted using a bench-scale collimated-beam UV apparatus (SI Figure S1) equipped with a 4-W low-pressure UV lamp emitting light predominantly at 254 nm (Philips Co., Netherlands). The emission spectrum (SI Figure S2) of the lamp was characterized by a spectroradiometer (Spectral Evolution, SR-1100). The reaction solution (10 mL) was poured into a sterile glass Petri dish (inner diameter = 5.4 cm) which was placed on a stir plate, perpendicular to the incident light. The UV fluence rate received in the reaction solution was measured to be 2.2×10^{-7} Einstein·L^{-1}·s^{-1} using potassium ferrioxalate chemical actinometry.[20] For the experiments conducted in a clean water matrix, phosphate buffer solution (3.0 mM PBS at pH 7.0) was applied by spiking with PAA (or H_2O_2) and *E. coli*, except where stated otherwise. Experiments related to the water matrix effect were conducted using secondary effluent (nondisinfected) from a municipal wastewater treatment facility located near Atlanta, Georgia. Three parameters of the wastewater effluent were measured to be pH 6.09, absorbance at 254 nm (UV_{254}) of 0.136, and chemical oxygen demand (COD) of 27.6 ppm before use. The pH of the effluent water was adjusted to 7.0 using NaOH. All the data reported herein were from two batches of experiments, in which the initial density of *E. coli* was around 1×10^8 CFU·mL^{-1} (Figures 1 and 2b and Figures S3–S6) or 5×10^6 CFU·mL^{-1} (Figure 2a and Figure S7). All experiments were at least duplicated, and standard deviations together with mean values of repeated experiments were reported in the figures.

RESULTS AND DISCUSSION

Inactivation of *E. coli* by UV, PAA, and UV/PAA. The comparison of *E. coli* inactivation under the different disinfection conditions of UV, PAA, and UV/PAA was studied. Figure 1 shows that 4-log inactivation of *E. coli* was achieved by UV alone or PAA alone at 4 min, while the same log of

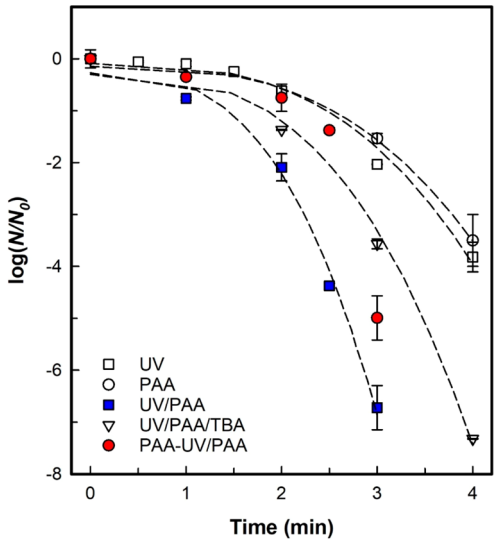

Figure 1. Inactivation of *E. coli* under UV disinfection (UV), PAA disinfection (PAA), PAA combined with UV (UV/PAA), UV/PAA with spiking of TBA (UV/PAA/TBA), and PAA 2 min then exposing under UV (PAA-UV/PAA). UV fluence rate = 2.2×10^{-7} Einstein·L^{-1}·s^{-1}, $[PAA]_0$ = 9 mg·L^{-1} (i.e., 0.12 mM), $[TBA]_0$ = 10 mM, and $[E. coli]_0 \sim 1 \times 10^8$ CFU·mL^{-1}. Symbols represent experimental observation, and dashed lines are fittings using the Hom Model. PAA-UV/PAA is not fitted by the model.

inactivation was achieved within 2.5 min when UV was combined with PAA. At 3 min, 7-log inactivation was achieved under UV/PAA conditions, which was significantly higher than those under UV alone or PAA alone conditions (i.e., less than 2-log inactivation). To better understand the mechanisms of different disinfection processes, UV and PAA disinfection were studied individually.

UV irradiation is effective to inactivate bacteria, primarily due to dimerization of adjacent thymine molecules in their DNA.[9] By plotting log-inactivation of *E. coli* with *CT* (concentration × time) values of UV irradiation (SI Figure S3), data obtained in this study is comparable with those previously reported.[10,12] Although PAA has not been extensively studied in the literature, the disinfection capability of PAA is expected to be resulted from its high oxidation power which allows it to oxidize sensitive sulfhydryl and sulfur bonds in proteins, enzymes, and other key biomolecules.[21] A log-inactivation vs *CT* curve for PAA is shown in SI Figure S4, which was obtained by varying both PAA concentrations (3–27 mg·L^{-1}) and contact time (0–10 min) in PBS at pH 7.0. Inactivation of *E. coli* by PAA alone had not been studied in PBS previously. In wastewater effluent, it was reported that 3-log inactivation required 30 to 120 mg·L^{-1}·min of PAA depending on water matrices.[2] Therefore, the approximate 33 mg·L^{-1}·min of PAA required for 3-log inactivation in this study (SI Figure S4) is in the lower end of previously reported range.

To investigate the role of free radicals, mainly hydroxyl radical ($^{\bullet}OH$) and acetyloxyl radicals (i.e., $CH_3C(=O)O^{\bullet}$) generated when PAA is exposed under UV, in the enhanced efficiency of *E. coli* inactivation, an excess amount of *tert*-butyl alcohol (TBA) was applied to quench $^{\bullet}OH$ under UV/PAA. As Figure 1 shows, at 3 min, there was 3.2-log less inactivation of *E. coli* under UV/PAA with the presence of TBA (denoted as UV/PAA/TBA) compared with that without TBA under UV/PAA, which confirmed the important role of $^{\bullet}OH$ in the

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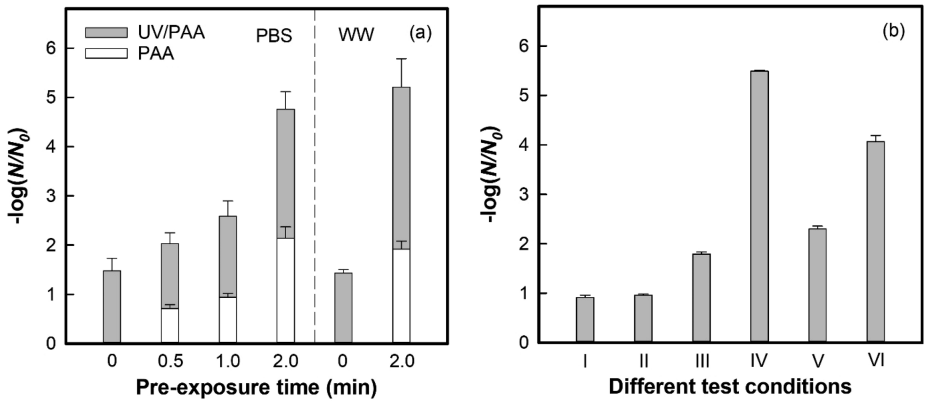

Figure 2. Inactivation of *E. coli* under different conditions. (a) *E. coli* exposed to PAA for different times (0–2.0 min) before applying UV/PAA for 1 min in PBS or in wastewater effluent; $[PAA]_0$ = 5 mg·L^{-1}, $[E.\ coli]_0 \sim 5 \times 10^6$ CFU·mL^{-1}. (b) *E. coli* inactivation after different treatments: (I) UV/H_2O_2 (1 min) + PAA (2 min); (II) PAA (2 min) + PAA removal + UV/H_2O_2 (1 min); (III) PAA (2 min) + PAA removal + UV/PAA (1 min); (IV) PAA (2 min) + UV/PAA (1 min); (V) TBA (5 min) + PAA (2 min) + UV/PAA/TBA (1 min); (VI) PAA (2 min) + UV/PAA/TBA (1 min); $[PAA]_0$ = 9 mg·L^{-1}, $[H_2O_2]_0$ = 4 mg·L^{-1}, $[TBA]_0$ = 10 mM, $[E.\ coli]_0 \sim 1 \times 10^8$ CFU·mL^{-1} (details of treatments listed in SI Text S5).

inactivation of *E. coli* under UV/PAA condition. Furthermore, the disinfection kinetics of UV, PAA, UV/PAA, and UV/PAA/TBA were fitted by the Hom Model[22] as eq 1 (dashed lines in Figure 1).

$$\log\left(\frac{N_0}{N}\right) = -k \cdot C \cdot t^3 \quad (1)$$

where k is the disinfection rate constant, C is the disinfectant concentration, and t is the elapsed time. A linear relationship ($R^2 > 0.989$) was observed for all experimental conditions when log survival was plotted versus t^3 (SI Figure S5). As Figure S5 shows, the slope of fitting using the Hom model under UV/PAA/TBA is equal to the summation of the slopes under UV alone and PAA alone conditions, suggesting that $^{\bullet}OH$ was critical, while the acetyloxyl radicals played a negligible role in increasing the inactivation efficiency for *E. coli*. The acetyloxyl radical could react with organics, dissociate to $CH_3^{\bullet}$ and CO_2, or react with PAA to form $CH_3C(=O)OO^{\bullet}$.[11]

To further assess the contribution of $^{\bullet}OH$, UV/PAA was compared to a more extensively studied advanced disinfection process, UV/H_2O_2, which produces $^{\bullet}OH$ as the major disinfectant. The experiments were designed to employ the same initial molar concentration (0.12 mM) of PAA and H_2O_2, respectively. In such cases, a higher steady-state concentration of $^{\bullet}OH$ is expected under UV/H_2O_2 than UV/PAA (calculation detailed in SI Text S4), which was confirmed by experiments using nitrobenzene as a $^{\bullet}OH$ probe compound and observing a faster degradation rate of nitrobenzene under UV/H_2O_2 than UV/PAA (data not shown). Judging by the slopes of the Hom model on the disinfection kinetic data, UV/H_2O_2 only moderately enhanced the inactivation of *E. coli* compared to UV only (Figures S6). The inactivation of *E. coli* by a low dose of H_2O_2 alone has been shown to be negligible previously.[12] UV/PAA, even with a lower $^{\bullet}OH$ concentration than UV/H_2O_2, enhanced *E. coli* inactivation much more significantly compared to UV alone or PAA alone (Figure S5). Therefore, $^{\bullet}OH$ produced under UV/PAA is not the only reason behind the enhanced inactivation of *E. coli*.

To better understand the enhancement of *E. coli* inactivation under UV/PAA, PAA and UV were applied sequentially to obtain insight. Under the experimental condition of PAA-UV/PAA in Figure 1, the *E. coli* were pre-exposed to PAA for 2 min before UV was introduced. A 4.2-log inactivation of *E. coli* occurred in just 1 min after UV was applied (i.e., within minute 2 and 3) which was 3.5-log inactivation higher than directly applying UV/PAA for 1 min (i.e., within minute 0 and 1) when the similar initial cell density was applied. Therefore, PAA-UV/PAA could be a promising new disinfection strategy and prompted further study to understand the mechanism.

Inactivation of *E. coli* by PAA-UV/PAA. To better understand how pre-exposure to PAA affect *E. coli* inactivation by UV/PAA, experiments were designed to pre-expose *E. coli* to PAA for different times (0–2 min), followed by application of UV for 1 min (Figure 2a). Although pre-exposure of *E. coli* to PAA in the dark caused some cell inactivation before UV application, the log inactivation of *E. coli* caused by UV/PAA (i.e., after UV was applied) was 1.48, 1.32, 1.64, and 2.62 with 0, 0.5, 1, and 2 min of pre-exposure time, respectively. Evidently, the longest pre-exposure time of 2 min led to the greatest *E. coli* inactivation by UV/PAA compared to without pre-exposure to PAA. The same procedure was adapted to evaluate inactivation of *E. coli* by H_2O_2–UV/H_2O_2 (i.e., pre-exposing *E. coli* to H_2O_2, followed by UV application). In contrast, no enhancement of *E. coli* inactivation by UV/H_2O_2 (i.e., after UV was applied) was observed by pre-exposing *E. coli* to H_2O_2 (Figure S7).

Two hypotheses may be used to explain the enhancement of *E. coli* inactivation by pre-exposing to PAA during the disinfection of UV/PAA: (1) After a certain period of exposure to PAA, *E. coli* become more vulnerable to the following UV/PAA process. (2) The pre-exposure allows PAA to diffuse into and/or adsorb on *E. coli*, and then $^{\bullet}OH$ produced in/on *E. coli* leads to a better cell inactivation compared with the radicals produced in the bulk solution.

Hypothesis (1) was tested by three conditions: (I) cells pretreated by UV/H_2O_2, followed by PAA, (II) cells pretreated by PAA, followed by PAA removal and then UV/H_2O_2, and (III) cells pretreated by PAA, followed by PAA removal and then UV/PAA (Figure 2b and Text S5). In conditions II and III, sodium thiosulfate was used to remove PAA after the pre-exposure, and then, H_2O_2 or PAA was (re)-introduced together with UV. The similar log inactivation of *E. coli* for conditions I and II suggests that pre-exposure to one of the two oxidants ($^{\bullet}OH$ and PAA) does not make the cells more vulnerable to the other oxidant. Furthermore, the inactivation of *E. coli* by condition III was found to be much lower than that by

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condition IV (cells pretreated by PAA, followed by UV/PAA without PAA's removal after the pre-exposure and reintroduction along with UV as in condition III). If pre-exposure to PAA did make *E. coli* more vulnerable to UV/PAA, condition III should exhibit similar inactivation efficiency as condition IV.

Hypothesis (2) was tested by directly monitoring PAA concentration in the bulk solution before and after spiking *E. coli* into the reactor. The same initial PAA concentration (5 mg·L^{-1}) and *E. coli* density (~5×10^6 CFU·mL^{-1}) as in Figure 2a were applied in the test, and 5% and 11% losses of PAA were observed in the bulk solution after 0.5 and 4 min, respectively. However, it is difficult to confirm whether the PAA loss was due to diffusion into/adsorption on *E. coli* or direct reaction with cells. To further test hypothesis (2), experiments were designed by spiking TBA at different stages to quench the $^{\bullet}OH$ produced in *E. coli* and bulk solution (Figure 2b, conditions V and VI). It is reasonable to assume that TBA behaves similarly as PAA in terms of diffusion into/adsorption on bacteria membranes due to the alkyl moiety. H_2O_2, on the other hand, is likely to have lower affinity for bacteria membranes due to its inorganic nature. Under condition V, *E. coli* suspensions were premixed with TBA for 5 min so that TBA diffused into *E. coli* cells and could quench $^{\bullet}OH$ inside *E. coli* cells when PAA and UV were introduced in the UV/PAA process. Under condition VI, UV and TBA were introduced at the same time after *E. coli* cells were premixed with PAA so that TBA was only present in the bulk phase of the *E. coli* suspension while PAA had adsorbed onto and/or entered into *E. coli* cells. The significant difference of inactivation between conditions V and VI (Figure 2b) clearly suggests that the strong disinfection power of $^{\bullet}OH$ was likely due to the location where $^{\bullet}OH$ was produced. Indeed, unlike H_2O_2, PAA bears an alkyl moiety which provides hydrophobic characteristics for PAA. Thus, PAA more easily adsorbs onto bacteria membranes and enters into cells.

Environmental Implications. As discussed above, two critical steps likely lead to the significant enhancement of bacteria inactivation by UV/PAA: adsorption/diffusion of PAA onto/into cells and UV activation of PAA. This study demonstrates that UV/PAA is an effective disinfection process and also that pre-exposure of *E. coli* to PAA followed by UV irradiation (i.e., the PAA-UV/PAA process) will achieve the most significant inactivation. Although inactivation kinetics may differ due to adsorption behavior of PAA likely relying on the properties of extracellular polymeric substances (EPS) of specific bacteria, similar enhanced inactivation may be expected. Under the experimental conditions in this study, a short pre-exposure time of 2 min to PAA before applying UV is sufficient to lead to significantly enhanced inactivation. Moreover, the same enhanced disinfection could be obtained in municipal wastewater in which 3.3 log inactivation of *E. coli* by UV/PAA was achieved with PAA pre-exposure, compared with 1.4 log inactivation without pre-exposure (Figure 2a). As the results showed 0.75−2 log inactivation by 2 min PAA pre-exposure and 2.6−4.2 log additional inactivation by UV/PAA during the minute when UV was applied, sequential application of PAA and UV achieved significant disinfection power with less energy input. Given the same amount of chemical cost (i.e., PAA concentration), the energy cost (from UV irradiation) per order of inactivation with the PAA-UV/PAA process (i.e., 2.6×10^{-6} Einstein·L^{-1}) is at least less than half of the cost with the UV/PAA process (i.e., 5.7×10^{-6} Einstein·L^{-1}). Therefore, it is reasonable to suggest that the UV/PAA process can be optimized by sequentially applying PAA and UV so that significant energy cost from UV irradiation can be saved.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.estlett.8b00249.

Text S1−S5 and Figures S1−S7. (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Luukkonen, T.; Heyninck, T.; Rämö, J.; Lassi, U. Comparison of organic peracids in wastewater treatment: Disinfection, oxidation and corrosion. *Water Res.* **2015**, *85*, 275−285.

(2) Biswal, B. K.; Khairallah, R.; Bibi, K.; Mazza, A.; Gehr, R.; Masson, L.; Frigon, D. Impact of UV and peracetic acid disinfection on the prevalence of virulence and antimicrobial resistance genes in uropathogenic escherichia coli in wastewater effluents. *Appl. Environ. Microbiol.* **2014**, *80*, 3656−3666.

(3) Ragazzo, P.; Chiucchini, N.; Piccolo, V.; Ostoich, M. A new disinfection system for wastewater treatment: Performic acid full-scale trial evaluations. *Water Sci. Technol.* **2013**, *67*, 2476−2487.

(4) Rossi, S.; Antonelli, M.; Mezzanotte, V.; Nurizzo, C. Peracetic acid disinfection: A feasible alternative to wastewater chlorination. *Water Environ. Res.* **2007**, *79*, 341−350.

(5) Koivunen, J.; Heinonen-Tanski, H. Peracetic acid (PAA) disinfection of primary, secondary and tertiary treated municipal wastewaters. *Water Res.* **2005**, *39*, 4445−4453.

(6) Kitis, M. Disinfection of wastewater with peracetic acid: A review. *Environ. Int.* **2004**, *30*, 47−55.

(7) Dell'Erba, A.; Falsanisi, D.; Liberti, L.; Notarnicola, M.; Santoro, D. Disinfection by-products formation during wastewater disinfection with peracetic acid. *Desalination* **2007**, *215*, 177−186.

(8) Holah, J.; Higgs, C.; Robinson, S.; Worthington, D.; Spenceley, H. A conductance-based surface disinfection test for food hygiene. *Lett. Appl. Microbiol.* **1990**, *11*, 255−259.

(9) Madigan, M. T.; Martinko, J. M.; Dunlap, P. V.; Clark, D. P. *Brock Biology of Microorganisms*, 12th ed; Pearson, 2008; pp 65−73.

(10) Hijnen, W.; Beerendonk, E.; Medema, G. J. Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo)cysts in water: A review. *Water Res.* **2006**, *40*, 3−22.

(11) Cai, M.; Sun, P.; Zhang, L.; Huang, C.-H. UV/peracetic acid for degradation of pharmaceuticals and reactive species evaluation. *Environ. Sci. Technol.* **2017**, *51*, 14217−14224.

(12) Sun, P.; Tyree, C.; Huang, C.-H. Inactivation of escherichia coli, bacteriophage MS2, and bacillus spores under UV/H_2O_2 and UV/peroxydisulfate advanced disinfection conditions. *Environ. Sci. Technol.* **2016**, *50*, 4448−4458.

(13) Cho, M.; Chung, H.; Choi, W.; Yoon, J. Linear correlation between inactivation of E. coli and OH radical concentration in TiO_2 photocatalytic disinfection. *Water Res.* **2004**, *38*, 1069−1077.
(14) Mamane, H.; Shemer, H.; Linden, K. G. Inactivation of E. coli, B. subtilis spores, and MS2, T4, and T7 phage using UV/H_2O_2 advanced oxidation. *J. Hazard. Mater.* **2007**, *146*, 479−486.
(15) Caretti, C.; Lubello, C. Wastewater disinfection with PAA and UV combined treatment: A pilot plant study. *Water Res.* **2003**, *37*, 2365−2371.
(16) Chhetri, R. K.; Klupsch, E.; Andersen, H. R.; Jensen, P. E. Treatment of Arctic wastewater by chemical coagulation, UV and peracetic acid disinfection. *Environ. Sci. Pollut. Res.* **2017**, *24*, 1−9.
(17) Lubello, C.; Caretti, C.; Gori, R. Comparison between PAA/UV and H_2O_2/UV disinfection for wastewater reuse. *Water Sci. Technol.: Water Supply* **2002**, *2*, 205−212.
(18) Koivunen, J.; Heinonen-Tanski, H. Inactivation of enteric microorganisms with chemical disinfectants, UV irradiation and combined chemical/UV treatments. *Water Res.* **2005**, *39*, 1519−1526.
(19) Federation, W. E. *Standard Methods for the Examination of Water and Wastewater*; American Public Health Association (APHA): Washington, DC, 2005.
(20) Harris, G. D.; Adams, V. D.; Moore, W. M.; Sorensen, D. L. Potassium ferrioxalate as chemical actinometer in ultraviolet reactors. *J. Environ. Eng.* **1987**, *113*, 612−627.
(21) Block, S. S. *Disinfection, Sterilization and Preservation*, 4th ed.; Lea & Febiger: Philadelphia, 1991.
(22) Gyurek, L. L.; Finch, G. R. Modeling water treatment chemical disinfection kinetics. *J. Environ. Eng.* **1998**, *124*, 783−793.

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Supporting Information

Rapid Disinfection by Peracetic Acid Combined with UV Irradiation

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Text S1. Chemicals and reagents

H_2O_2 (wt. 30%) was purchased from Fisher Scientific Co. (Pittsburgh, PA). Tertiary butyl alcohol (TBA) (99.5%) was purchased from Sigma Aldrich Inc. (St. Louis, MO). $Na_2S_2O_3$, Na_2HPO_4 and NaH_2PO_4 were obtained from Fisher Scientific Co. Difco™. Nutrient broth was obtained from Becton Dickinson (Franklin Lakes, NJ), Eosin methylene blue (EMB) agar was obtained from Sigma Aldrich Inc. (St. Louis, MO), and bacteriological agar was obtained from VWR International Inc. (West Chester, PA). Sterile phosphate buffered saline (pH 7.2) was obtained from Sigma Aldrich Inc. (St. Louis, MO). Freeze-dried *E. coli* culture (ATCC 15597) was purchased from ATCC and revived accordingly.

Text S2. Determination of PAA

The commercial PAA stock solution (~39% PAA, ~6% H_2O_2 and ~45% acetic acid by weight) was stored at 5 °C and regularly calibrated using titration methods. The sum of PAA and H_2O_2 concentration was first measured with the indirect iodometric titration, by adding potassium iodide (with ammonium molybdate as a catalyst) to produce the liberated iodine and then titrating the iodine with sodium thiosulfate. Then, the concentration of H_2O_2 in PAA solution was titrated with potassium permanganate under acidic pH. The PAA concentration in stock solution was obtained by subtracting H_2O_2 concentration from the sum concentration of PAA and H_2O_2.

The PAA working solution at ~9 $g \cdot L^{-1}$ was prepared weekly based on the concentration of PAA stock solution determined by the above titration method through appropriate dilution and stored at 5 °C. The residual PAA concentration in experiments was quantified by the *N,N*-diethyl-*p*-phenylenediamine (DPD) colorimetric method according to the Standard Methods 4500-Cl G.[1] The sample was treated with an excess amount of potassium iodide. The iodide was oxidized by PAA to produce iodine and iodine could react with DPD to form a pink colored species, which could be measured by absorbance at 515 nm and was in direct proportion to PAA concentration. Supplementary experiments confirmed that the low concentrations (< 2.5 $mg \cdot L^{-1}$) of H_2O_2 in the samples had negligible influence on PAA determination by the DPD method.

Text S3. Preparation of the culture stock of *E. coli* and experimental setup

Culture preparation followed the methods by Cho et al.[2] *E. coli* (ATCC 15597) was inoculated in nutrient broth (BD Difco^{TM} Nutrient broth) and grown for 18 h at 37°C. The culture was then transferred into a 50-mL tube, which was centrifuged at 1,000 g for 10 min. The supernatant was discarded. The bacteria palette was washed two more times, first with 50 mL of phosphate buffered saline and then with phosphate-buffered solution (PBS) (3 mM phosphate at pH 7). Stock suspension of *E. coli* was prepared by resuspending the final pellet in 2 mL PBS and stored at 4 ºC prior to use. To achieve an initial density of *E. coli* of around 1×10^8 CFU·mL^{-1} for each disinfection experiment, the stock suspension of *E. coli* was spiked in reaction solution by a dilution factor of 100–1000. Disinfection experiments were initiated by placing the petri dish (containing 3.0 mM PBS (pH 7), PAA and *E. coli*,) under UV exposure. Preliminary test showed that the pH was stable during the disinfection experiments. For each condition, at least triplicate experiments were conducted. For each sample, a ten-fold serial dilution was performed up to 1/10,000 dilution ratio using 30 mM PBS at pH 7.1. Residue PAA and/or H_2O_2 were removed by addition of an excess amount of sodium thiosulfate. The amount of 0.1-mL aliquot of each diluted solution was inoculated onto each of three replicates of 47-mm sterile Petri dishes containing nutrient agar (8 g·L^{-1} nutrient broth + 15 g·L^{-1} agar). Colony forming units (CFU) were counted after incubation at 37 ºC for 24 h.

A second set of experiments was performed to further test the hypothese proposed in this study. Here, the cells were kept in constant growth by changing the broth every 35 hours (2 mL of incubated broth with *E. coli* was transferred to 35 mL of sterile nutrient broth every 35 hours until all experiments were performed). Cell washing proceeded as stated above. Most experimental parameters were kept the same as those in the first set of experiments except the cell density, which was around 5×10^6 CFU·mL^{-1} for the second set of experiments. For experiments performed using DI water, BD Difco^{TM} nutrient broth with agar (with the same proportions as the ones made for the first set of experiments) was used to cultivate the *E. coli*. 0.1 mL of samples were taken from the disinfection reactor and quenched with $Na_2S_2O_3$ before doing base 10 serial dilutions. Between 0.05 to 0.1 mL of samples were plated depending on the dilution factor and the exposure time of the experiments to facilitate CFU counting..

Wastewater was obtained from the secondary effluent (non-disinfected) of a municipal

wastewater treatment facility located near Atlanta, Georgia and was used in experiments to assess the matrix effect on disinfection. Due to the nature of the water, a selective and differential culture medium for *E. coli* was chosen (EMB) to perform the plating for these experiments. The medium was prepared using 37.5 g of medium in 1 liter of DI water. After autoclaving for 121 °C for 15 min, the medium was poured on the plates and stored at 4 °C until used. CFUs were counted after an incubation period of 18-24 h at 37 °C.

All experiments were conducted in conjunction with negative controls consisting of only DI water, PBS, and/or wastewater and following the same experimental procedures. Furthermore, in order to rule out potential plate contamination, two non-plated petri dishes with medium (Nutritive agar or EMB agar) were also incubated for comparison.

Text S4. Estimation of hydroxyl radical concentration under UV/PAA condition

The steady-state concentration of hydroxyl radical (•OH) in PBS under UV/PAA condition at pH 7 can be estimated using the eqn (S1) as described previously:[3]

$$[^{\bullet}OH]_{ss} = \frac{\text{Generation rate}}{\text{Consuming rate}} = \frac{2.303 \cdot \varepsilon_{PAA} \cdot I \cdot \Phi_{PAA} \cdot l \cdot [PAA] + 2 \cdot 2.303 \cdot \varepsilon_{H_2O_2} \cdot I \cdot \Phi_{H_2O_2} \cdot l \cdot [H_2O_2]}{k_{PAA} \cdot [PAA] + k_{H_2O_2} \cdot [H_2O_2] + k_{acetate} \cdot [CH_3COO^-]} \quad \text{(S1)}$$

where I is the UV fluence rate (2.2×10^{-7} Einstein·L^{-1}·s^{-1}), ε is the molar absorbance of PAA and H_2O_2 at pH 7.0 (ε_{PAA} = 10.01 $mole^{-1}$·cm^{-1}, ε_{H2O2} = 19 $mole^{-1}$·cm^{-1}), Φ is the quantum yield of PAA and H_2O_2 at pH 7.0 (Φ_{PAA} = 1.2 mole·$Einstein^{-1}$, Φ_{H2O2} = 0.5 mole·$Einstein^{-1}$),[3] l is the light path length (~0.4 cm), *k* represents the second-order rate constants between hydroxyl radical and PAA (k_{PAA} = 9.33×10^8 $M^{-1}s^{-1}$), H_2O_2 (k_{H2O2} = 2.7×10^7 $M^{-1}s^{-1}$) and acetate ($k_{acetate}$ = 8.5×10^7 $M^{-1}s^{-1}$).[3] In the pH 7 PBS solution containing 9 mg·L^{-1} PAA, most PAA was protonated (pKa = 8.2)[4] at 0.12 mM and most acetic acid was deprotonated at around 0.17 mM. The concentration of H_2O_2 was 1.38 mg/L (i.e. 0.04 mM). When substituting all known values into the eqn (S1), the steady-state hydroxyl radical concentration was estimated to be 3.5×10^{-15} M.

The steady-state concentration of hydroxyl radical in PBS under UV/H_2O_2 condition at pH 7 can also be estimated using eqn (S1) but without the components of PAA and acetic acid. At

the dose of 0.12 mM H_2O_2, the estimated steady-state hydroxyl radical concentration is around 2.9×10^{-13} M.

Text S5. Experimental procedures for tested conditions

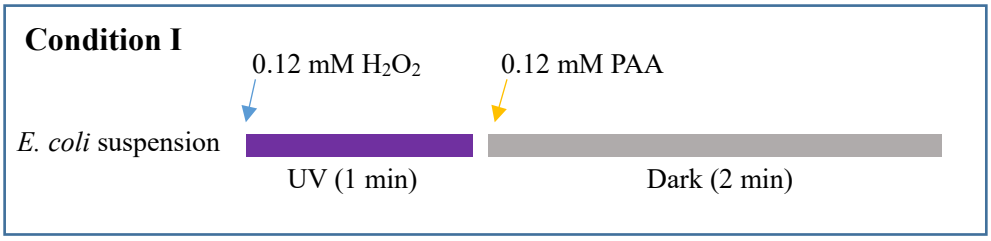
In Figure 1 and Figure 2a:

- **UV alone.** *E. coli* suspension (~ 1×10^8 CFU) was prepared in PBS at pH 7.0, and then exposed to UV irradiation. Aliquots were sampled periodically throughout the 4-min disinfection. CFU numbers were then obtained with methods described in Text S3.
- **PAA alone.** *E. coli* suspension (~ 1×10^8 CFU) was prepared in PBS at pH 7.0, and then spiked with 9 or 5 $mg\cdot L^{-1}$ (i.e., 0.12 or 0.067 mM) PAA. Aliquots were sampled periodically throughout the 4-min disinfection. The residual PAA was decomposed by addition of an excess amount (1 mM) of sodium thiosulfate. CFU numbers were then obtained with methods described in Text S3.
- **UV/PAA.** *E. coli* suspension (~ 1×10^8 CFU) in PBS at pH 7.0 was spiked with 9 or 5 $mg\cdot L^{-1}$ (i.e., 0.12 or 0.067 mM) PAA, and then exposed to UV irradiation immediately. Aliquots were sampled periodically throughout the 4-min disinfection. The residual PAA was decomposed by addition of an excess amount (1 mM) of sodium thiosulfate. CFU numbers were then obtained with methods described in Text S3.
- **UV/PAA/TBA.** *E. coli* suspension in PBS at pH 7.0 was spiked with 9 $mg\cdot L^{-1}$ PAA and 10 mM TBA, and then exposed to UV irradiation immediately. Aliquots were sampled periodically throughout the 4-min disinfection. The residual PAA was decomposed by addition of an excess amount (1 mM) of sodium thiosulfate. CFU numbers were then obtained with methods described in Text S3.
- **PAA-UV/PAA.** *E. coli* suspension in PBS at pH 7.0 was spiked with 9 or 5 $mg\cdot L^{-1}$ PAA. After 0.5-2 minutes of contact time, the suspension was exposed to UV irradiation, which created UV/PAA condition. After one minute of treatment, the suspension was removed from UV light. The residual PAA was decomposed by an excess amount of sodium thiosulfate. CFU numbers were then obtained with methods described in Text S3.

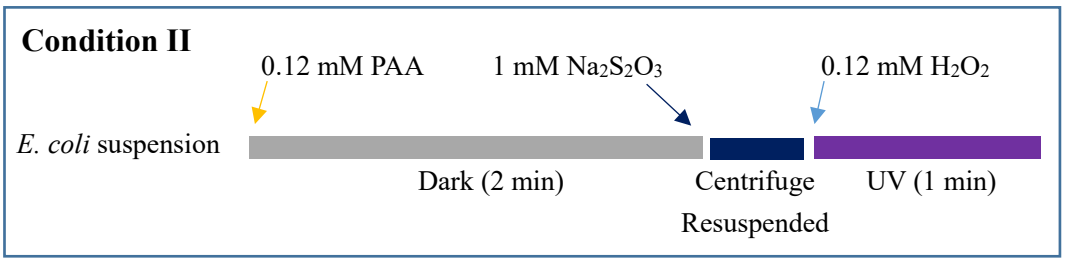
In Figure 2b:

- **Condition I** (UV/H_2O_2 for 1 min, then exposed to PAA in dark for 2 min). *E. coli*

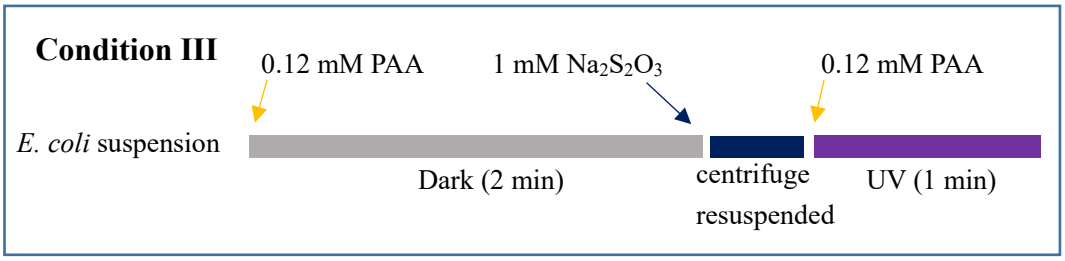
suspension in PBS at pH 7.0 was spiked with 0.12 mM H_2O_2, then exposed to UV irradiation immediately. After one minute, the suspension was then removed from UV light and spiked with 9 mg·L^{-1} (i.e., 0.12 mM) PAA. After two minutes, the residue H_2O_2 and PAA were decomposed by addition of an excess amount (1 mM) of sodium thiosulfate. CFU numbers were then obtained with methods described in Text S3.



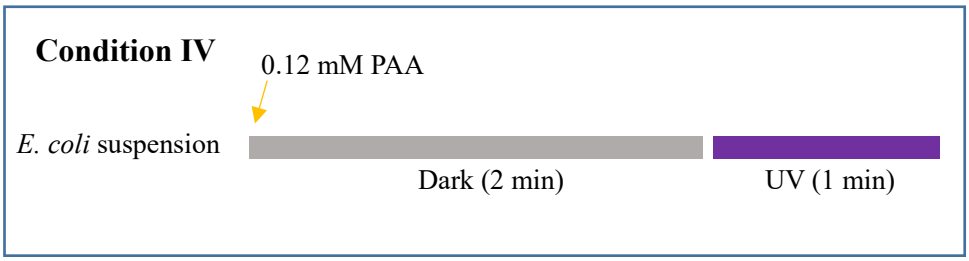
- **Condition II** (PAA for 2 min, then treated with $Na_2S_2O_3$, then exposed to UV/H_2O_2 for 1 min). *E. coli* suspension in PBS at pH 7.0 was spiked with 9 mg·L^{-1} PAA. After two minutes of contact time, PAA was removed by an excess amount (1 mM) of sodium thiosulfate. The suspension was then centrifuged at 1000 g twice and the supernatant was discarded. The *E. coli* pellet was collected and resuspended in PBS at pH 7.0 with 0.12 mM H_2O_2. Then, the suspension was exposed to UV irradiation for one minute. The residue H_2O_2 was decomposed by an excess amount of sodium thiosulfate. CFU numbers were then obtained with methods described in Text S3.



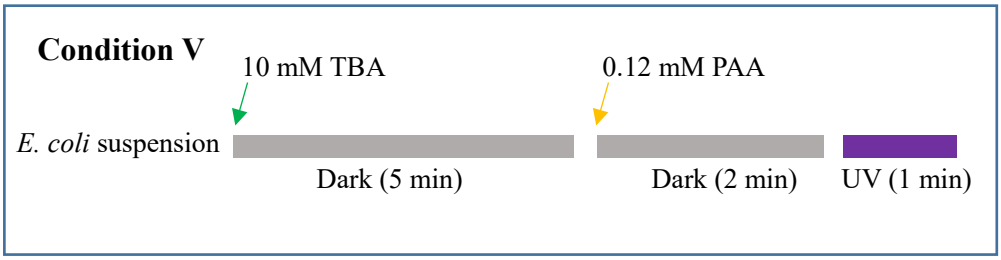
- **Condition III** (PAA for 2 min, then treated with $Na_2S_2O_3$, then exposed to UV/PAA for 1 min). Similar to condition I, *E. coli* suspension was treated with PAA for two minutes and then PAA was removed by sodium thiosulfate (1 mM). The suspension was centrifuged at 1000 g twice and the supernatant was discarded. The *E. coli* pellet resuspended in PBS at pH 7.0 with 9 mg·L^{-1} PAA was immediately exposed to UV irradiation for one minute. Then, the residue PAA was decomposed by an excess amount of sodium thiosulfate. CFU numbers were then obtained with methods described in Text S3.



- **Condition IV** (PAA in dark for 2 min, then UV/PAA for 1 min). *E. coli* suspension in PBS at pH 7.0 was spiked with 9 mg·L^{-1} PAA. After two minutes of contact time, the suspension was directly placed inside the UV reactor, which created UV/PAA condition. After one minute of treatment, the suspension was removed from UV light. The residue PAA was decomposed by an excess amount of sodium thiosulfate. CFU numbers were then obtained with methods described in Text S3.

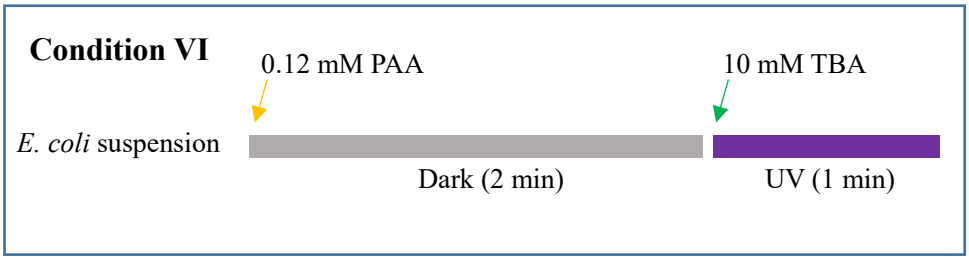


Condition V (TBA for 5 min, then PAA for 2 min, then UV/PAA/TBA for 1 min). *E. coli* suspension in PBS at pH 7.0 was pre-exposed to 10 mM TBA for five minutes. Then, PAA was spiked at 9 mg·L^{-1}. After two minutes of contact with PAA in dark, the suspension was exposed to UV irradiation for one minute. The residual PAA was decomposed by an excess amount of sodium thiosulfate. CFU numbers were then obtained with methods described in Text S3.



- **Condition VI** (PAA for 2 min, then UV/PAA/TBA for 1 min). *E. coli* suspension in PBS at pH 7.0 was spiked with 9 mg·L^{-1} PAA. After two minutes of contact time, the suspension

was mixed with 10 mM TBA and immediately transferred to the UV reactor. After one minute of treatment, the suspension was removed from UV light. The residual PAA was decomposed by an excess amount of sodium thiosulfate. CFU numbers were then obtained with methods described in Text S3.



- **Control Experiments.** Control experiments were conducted using *E. coli* suspension in PBS at pH 7.0 with addition of 10 mM TBA or with an excess amount (1 mM) of sodium thiosulfate. CFU numbers were obtained with methods described in Text S3 and compared to those without chemical addition. Results confirmed that TBA or sodium thiosulfate alone did not affect the *E. coli* cells within 5 minutes of contact time.

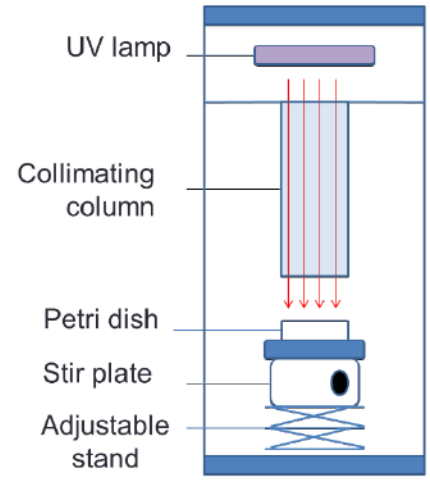


Figure S1. Illustration of collimated beam photoreactor.

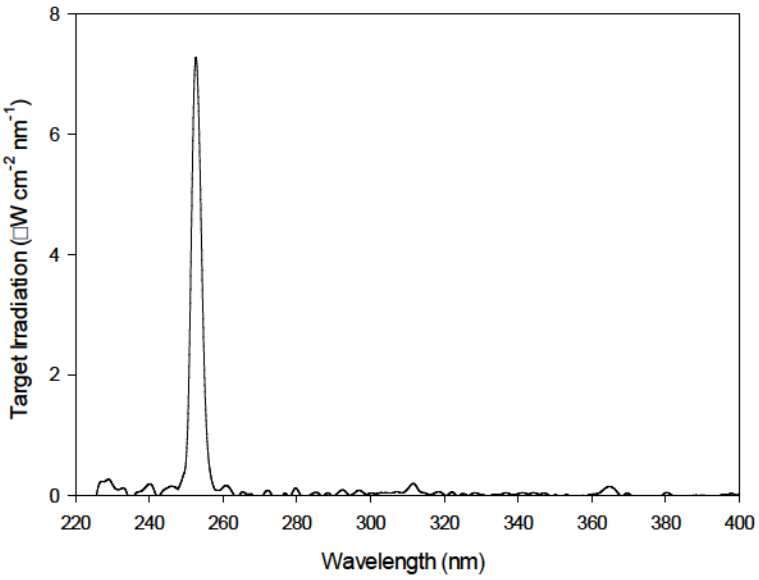


Figure S2. Light spectra of low pressure UV lamp.

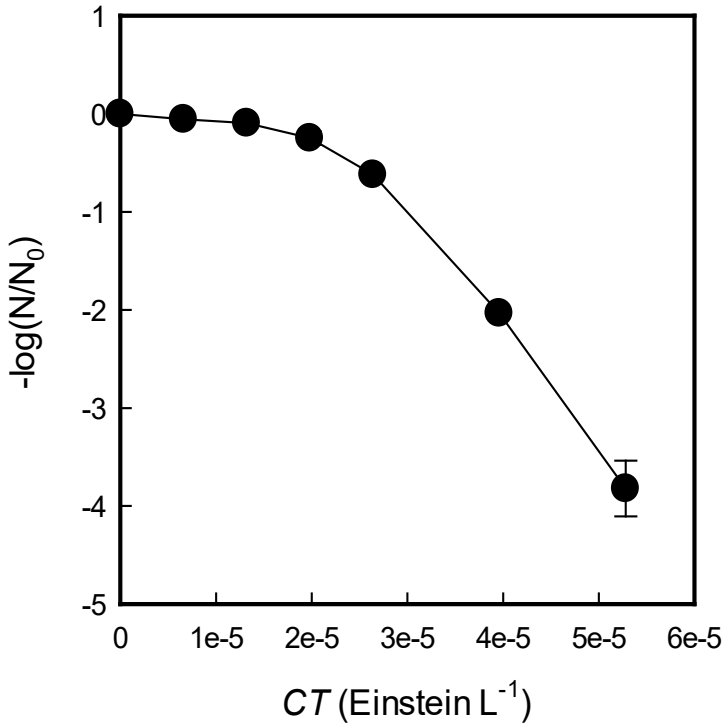


Figure S3. Inactivation of *E. coli* versus *CT* values of UV irradiance.

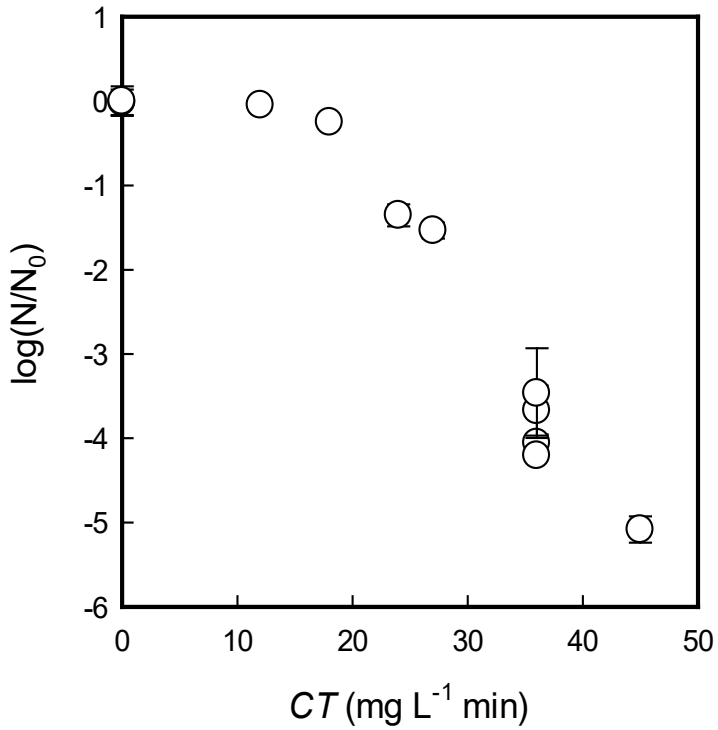


Figure S4. Inactivation of *E. coli* versus *CT* values of PAA.

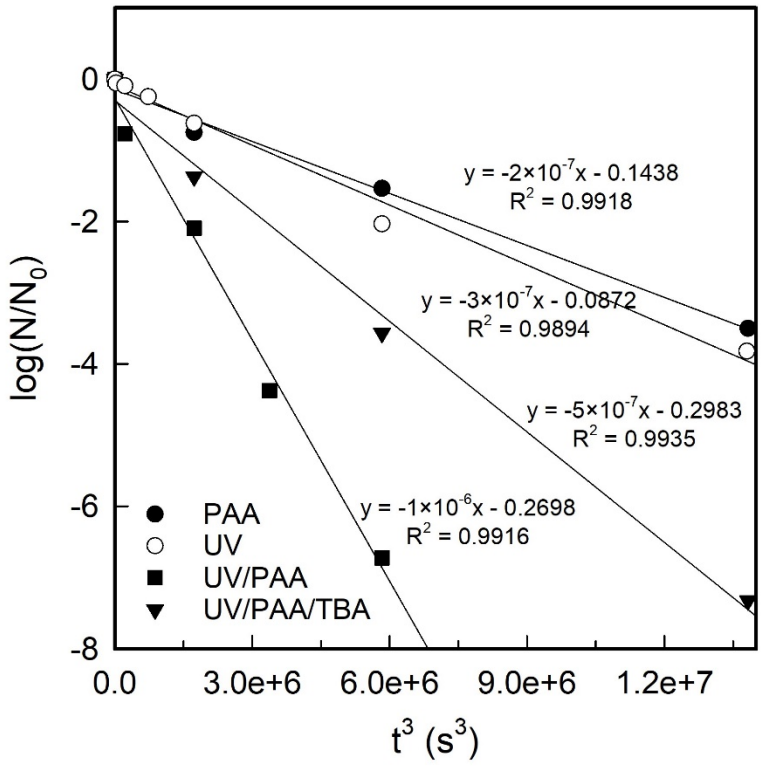


Figure S5. Inactivation of *E. coli* under PAA, UV, UV/PAA, and UV/PAA/TBA. Solid lines: Hom Model fitting under each condition by plotting log survival of *E. coli* versus t^3.

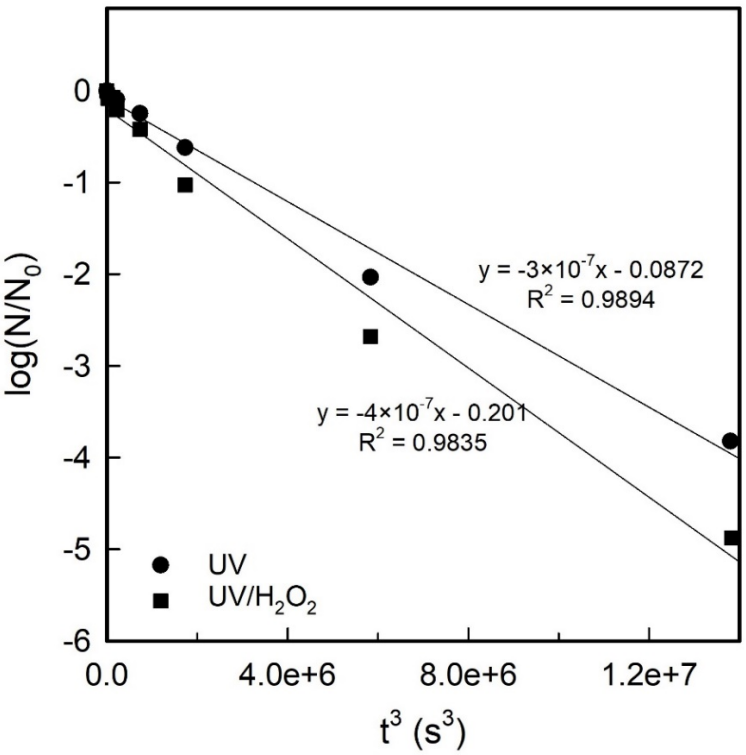


Figure S6. Inactivation of *E. coli* under UV and UV/H_2O_2. Solid lines: Hom Model fitting under each condition by plotting log survival of *E. coli* versus t^3.

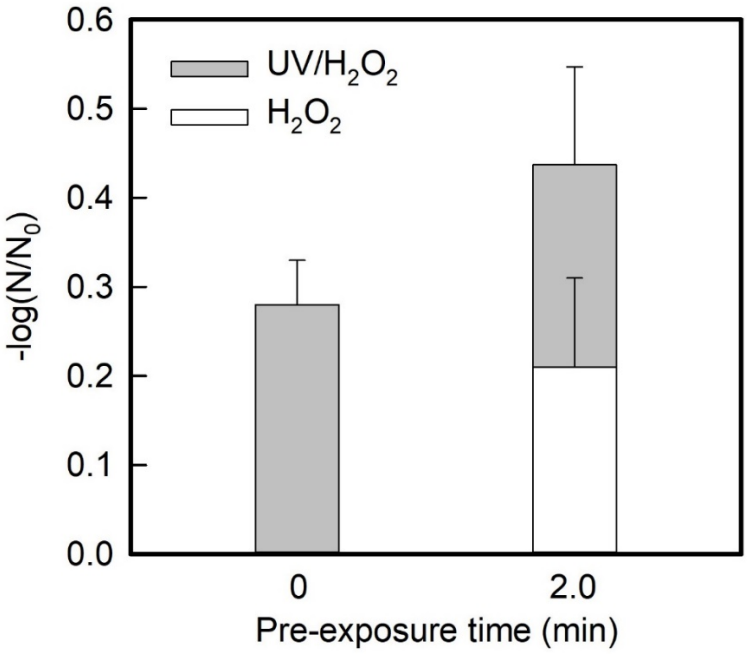


Figure S7. Inactivation of *E. coli* under different conditions. *E. coli* were exposed under H_2O_2 for 0 and 2 min before applying UV/H_2O_2 for 1 min in PBS.

REFERENCES

1. APHA-AWWA-WEF. Standard Methods for the Examination of Water and Wastewater, 20th ed.; American Public Health Association/American Water Works Association/Water Environment Federation: Washington DC, 1998.

2. Cho, M.; Gandhi, V.; Hwang, T.-M.; Lee, S.; Kim, J.-H. Investigating synergism during sequential inactivation of MS-2 phage and Bacillus subtilis spores with UV/H_2O_2 followed by free chlorine. *Water Res.* **2011,** *45*, 1063-1070.

3. Cai, M.; Sun, P.; Zhang, L.; Huang, C.-H. UV/Peracetic acid for degradation of pharmaceuticals and reactive species evaluation. *Environ. Sci. & Technol.* **2017**, 51, 14217-14224.

4. Yuan, Z.; Ni, Y.; Heiningen, A. R. P. V. Kinetics of peracetic acid decomposition: Part I: Spontaneous decomposition at typical pulp bleaching conditions. *Can. J. Chem. Eng.* **2010,** *75*, 37-41.