Environmental Science & Technology Letters

Letter

pubs.acs.org/journal/estlcu

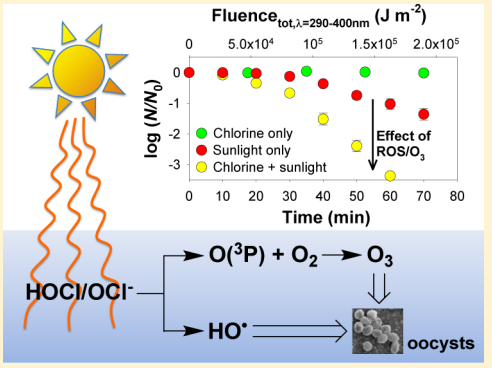
Enhanced Inactivation of *Cryptosporidium parvum* Oocysts during Solar Photolysis of Free Available Chlorine

Peiran Zhou,[†] George D. Di Giovanni,[§] John S. Meschke,[†,‡] and Michael C. Dodd*[,†,‡]

[†]Department of Civil & Environmental Engineering, University of Washington, Seattle, Washington 98195, United States
[‡]Department of Environmental & Occupational Health Sciences, University of Washington, Seattle, Washington 98195, United States
[§]The University of Texas-Houston School of Public Health, El Paso Regional Campus, El Paso, Texas 79902, United States

Supporting Information

ABSTRACT: Solar irradiation of aqueous solutions containing free available chlorine (FAC) dramatically enhances inactivation of *Cryptosporidium parvum* oocysts compared to FAC or sunlight alone. In pH 8, 10 mM phosphate buffer at 25 °C, exposure to FAC alone yields no oocyst inactivation at $CT_{FAC} \leq 832$ (mg min) L^{-1}, while exposure to simulated sunlight alone for 60 min yields <0.5 log inactivation. In contrast, exposure to simulated sunlight for 60 min in the presence of $[FAC]_0 = 8$ mg L^{-1} as Cl_2 results in photolytic decomposition of FAC to ~1 mg L^{-1} as Cl_2 [yielding $CT_{FAC} \sim 200$ (mg min) L^{-1}] accompanied by >2 log oocyst inactivation. Similar enhancement effects are observed in natural water under natural sunlight. Experiments undertaken in the presence of the reactive oxygen species (ROS) scavenger *tert*-butanol or in the absence of oxygen indicate that these enhancements are due to in situ ROS and ozone production via FAC photolysis.



INTRODUCTION

Cryptosporidiosis, a mild to severe gastrointestinal illness resulting from ingestion of viable oocysts of the protozoan parasite *Cryptosporidium*, is one of the most prevalent waterborne diseases worldwide.[1,2] Typical cryptosporidiosis symptoms include watery diarrhea, vomiting, dehydration, and weight loss. Although most individuals recover without treatment after 1−2 weeks, cryptosporidiosis may result in life-threatening symptoms in immunocompromised patients.[3]

Of the ~25 known *Cryptosporidium* species, *Cryptosporidium parvum* and *Cryptosporidium hominis* are most often responsible for human infections.[3,4] Oocysts of *C. parvum and C. hominis* appear to enter water supplies primarily through agricultural runoff and municipal wastewater effluent.[1,4−7] They are quite stable in freshwater and can persist in an infective state for weeks to months.[1,2,8] As few as ~10 viable oocysts may be sufficient to result in illness following ingestion, meaning even relatively "clean" waters can pose an infection risk.[1,3,8]

Because of the high chemical resistance of the oocyst wall, *Cryptosporidium* oocysts can survive even the highest levels of free available chlorine (FAC) exposure applied in conventional drinking water treatment,[9,10] often necessitating the use of alternative disinfection strategies employing ozone (O_3), ultraviolet (UV) light, or chlorine dioxide.[10−15] Considering this, it is noteworthy that O_3 and the potent oxidant hydroxyl radical ($HO^•$) can actually be generated in situ through photolysis of FAC (typically comprising HOCl and OCl^-) at the near-UV wavelengths characteristic of natural sunlight,[16−19] according to eqs 1−3[20]

$$HOCl/OCl^- + h\nu \rightarrow HO^•/O^{•-} + Cl^• \quad (1)$$

$$OCl^- + h\nu \rightarrow O(^3P) + Cl^- \quad (2)$$

$$O(^3P) + O_2 \rightarrow O_3 \quad k_3 = 4.0 \times 10^9\ M^{-1}\ s^{-1}\ ^{20} \quad (3)$$

with $Cl^•$ yielding additional $HO^•$ through reaction with H_2O.[21] As sunlight and $HO^•$ are also capable of inactivating *Cryptosporidium* oocysts,[22−25] it seems likely that solar FAC photolysis could provide an effective means of achieving oocyst inactivation without the specialized infrastructure required to utilize O_3 or other alternative disinfectants. Such an approach could be especially suited to small decentralized or point-of-use water treatment applications.

Recent findings confirm that sunlight-driven FAC photolysis can dramatically accelerate inactivation of highly chlorine resistant *Bacillus subtilis* endospores,[19] which are commonly used as surrogates for *Cryptosporidium* oocysts.[26−28] The present investigation was undertaken to evaluate the effectiveness of this approach for inactivating *C. parvum* oocysts themselves, using a cell culture immunofluorescence microscopy assay (CC-IFA) to quantify infectivity loss during exposure of FAC-containing solutions to simulated and natural sunlight.

Received: August 31, 2014
Revised: October 6, 2014
Accepted: October 7, 2014
Published: October 7, 2014

   dx.doi.org/10.1021/ez500270u | Environ. Sci. Technol. Lett. 2014, 1, 453−458

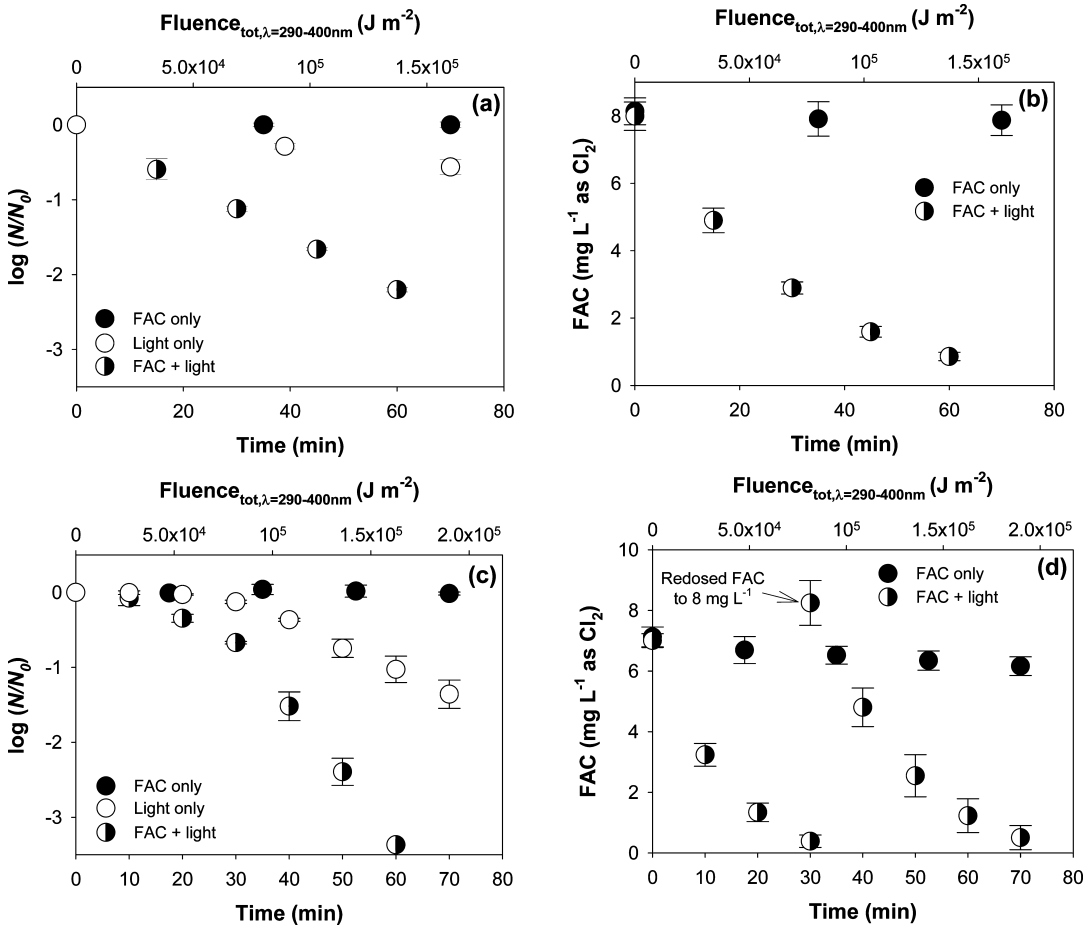


Figure 1. Enhancement of oocyst inactivation during FAC photolysis under simulated and natural sunlight. (a) Infectivity of *C. parvum* Iowa strain (Harley Moon) oocysts and (b) residual FAC concentrations in FAC-containing solutions of pH 8.0, 10 mM phosphate buffer at 25 °C during exposure to simulated sunlight. (c) Infectivity of *C. parvum* Iowa strain (Harley Moon) oocysts and (d) residual FAC concentrations in FAC-containing solutions of pH 8.0, 10 mM phosphate-buffered Tolt River water at ambient temperature (34 ± 2 °C) during exposure to natural sunlight. Fluence values pertain only to irradiated samples. See Table S2 (experimental sets 1 and 2) and Text S3 of the Supporting Information for details on the oocyst stocks and reactor configurations used in each experiment. Note that natural sunlight experiments, performed to evaluate the potential for applying FAC photolysis in a point-of-use SODIS process under ambient conditions, were undertaken in unthermostated quartz tubes on the roof of the More Hall Civil and Environmental Engineering building (Seattle, WA) at midafternoon on July 10, 2014, under clear skies.

MATERIALS AND METHODS

Chemicals and Materials. All reagents and growth media were purchased from commercial suppliers and were of at least reagent-grade purity. Ultrapure water (≥18.2 MΩ cm) from a Milli-Q Ultrapure system (Millipore) was used to prepare all solutions unless otherwise noted. Natural surface water was obtained from Seattle Public Utilities' Tolt River water treatment plant (Text S1 and Table S1 of the Supporting Information). FAC stocks prepared from sodium hypochlorite (5%, J. T. Baker) were standardized spectrophotometrically at λ = 292 nm ($\varepsilon_{292\ nm,OCl^-}$ = 350 M^{-1} cm^{-1}).[29] FAC concentrations were monitored during experiments using DPD colorimetry.[30] All reagents other than disinfectants and commercially available presterilized growth media were autoclaved or filter-sterilized prior to use.

***C. parvum* Oocyst Stock Preparation and Infectivity Assay.** Freshly shed oocysts of *C. parvum* Iowa isolate (Harley Moon)—passaged through mice, prepurified to 99% by Percoll–sucrose gradients, and suspended in phosphate-buffered saline amended with ampicillin and 0.01% Tween 20—were purchased from Waterborne Inc. (catalog no. P102M) and stored at 4 °C prior to use. Appropriate amounts of oocyst stock were centrifuged and washed with HyClone water (Thermo Scientific) three times before use in experiments. Additional information about oocyst stock handling and age is provided in Table S2 of the Supporting Information.

Oocyst infectivity was quantified using CC-IFA with HCT-8 human ileocecal adenocarcinoma cells (ATCC CCL-244).[31−34] General assay procedures were as described previously,[33] with the HCT-8 cells maintained according to procedures described in Text S2 of the Supporting Information. Briefly, oocyst samples were treated with 1% (w/v) trypsin in acidified Hanks' balanced salt solution (pH 2) at 37 °C for 1 h. Oocysts were then centrifuged, resuspended in IFA inoculation medium,[33] added to 48-well plates containing HCT-8 cell monolayers, and incubated at 5% CO_2 and 36 °C for 72 h. Plates were then fixed with methanol and stained with fluorescein-labeled rat antibodies specific for *C. parvum* Iowa isolate sporozoites (Sporo-Glo, Waterborne, Inc.). Infectious oocysts were enumerated with an inverted epifluorescence microscope (Text S2 of the Supporting Information). Samples were analyzed undiluted, 10-fold diluted, and 100-fold diluted, with dilutions prepared in pH 8, 10 mM phosphate buffer.

dx.doi.org/10.1021/ez500270u | *Environ. Sci. Technol. Lett.* 2014, 1, 453−458

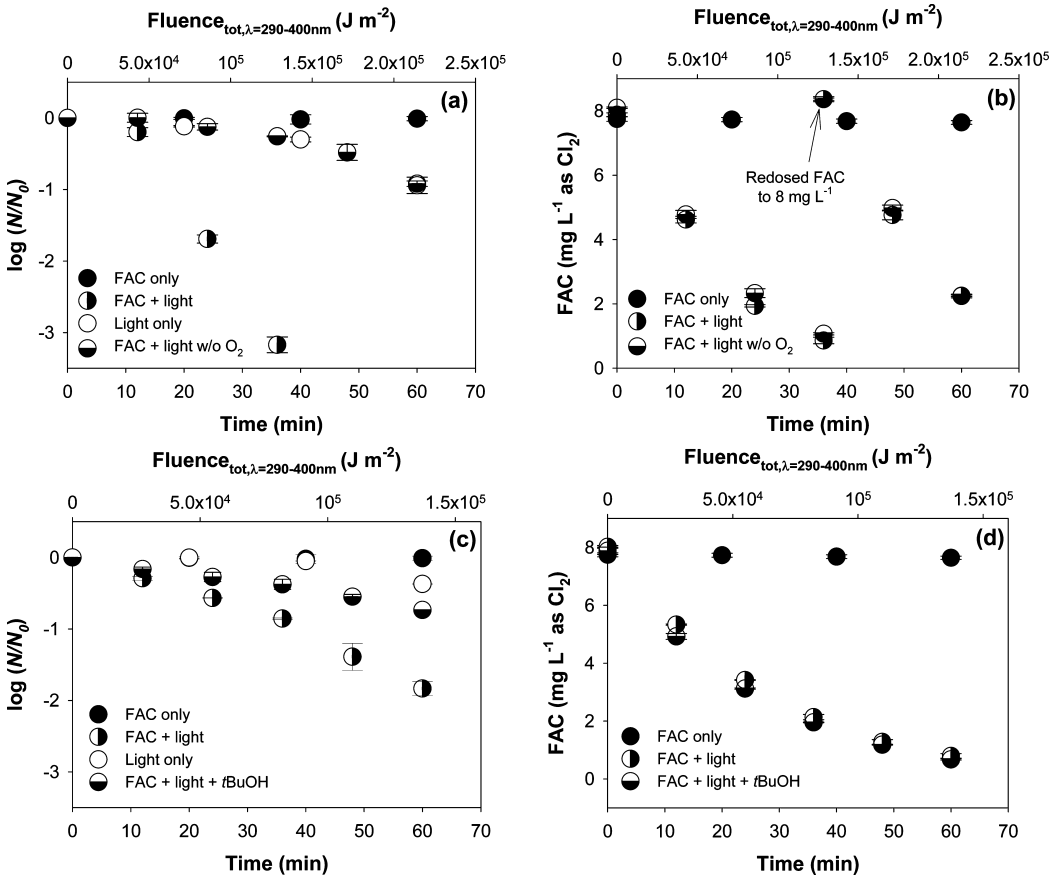


Figure 2. Influence of simulated solar irradiation on the infectivity of *C. parvum* Iowa strain (Harley Moon) oocysts in FAC-containing solutions of pH 8.0, 10 mM phosphate buffer at 25 °C in (a) the absence of O_2 and (c) the presence of 50 mM *t*BuOH. Panels b and d depict the corresponding variations in FAC concentrations under each condition. Fluence values pertain only to irradiated samples. See Table S2 (experimental sets 3 and 4) and Text S3 of the Supporting Information for details on the oocyst stocks and reactor configurations used in each experiment. Note that the accelerated kinetics of oocyst inactivation and FAC decay under FAC+light conditions in panels a and b are due to the configuration of the 300 mL photoreactor utilized for anoxic experiments (for details, see ref 19). The configuration of this reactor placed the water surface of the irradiated sample ~10 cm closer to the light source than in experiments undertaken in 50 mL crystallization dishes, resulting in a substantial increase in the effective fluence rate to which the sample was subjected (from 38 W m^{-2} in crystallization dishes to 60 W m^{-2} in the 300 mL reactor), which likely resulted in an increase in the in situ levels of ROS and O_3 generated during FAC photolysis.

Disinfection Procedures. All experiments were conducted in duplicate, using 10 mM phosphate buffer (pH 8) or 10 mM phosphate-buffered Tolt River water (pH 8), amended with ~5 × 10^4 oocysts mL^{-1}. Treatment was undertaken according to the following approaches: (i) exposure to FAC alone ("FAC only"), (ii) exposure to simulated or natural sunlight alone ("Light only"), (iii) exposure to simulated or natural sunlight in the presence of FAC ("FAC+light"), and (iv) exposure to simulated sunlight in the presence of (a) FAC without oxygen ("FAC+light w/o O_2") or (b) FAC with 50 mM *tert*-butanol ("FAC+light +*t*BuOH"). In general, experimental procedures and reactor configurations were as reported previously,[19] with minor variations (see Text S3 of the Supporting Information). All simulated sunlight experiments were conducted indoors and thermostated at 25 °C. Natural sunlight experiments, undertaken to evaluate the use of FAC photolysis under conditions representative of point-of-use solar disinfection (SODIS) applications,[35] were performed on the roof of the More Hall Civil and Environmental Engineering building without thermostating, at an ambient temperature of 34 ± 2 °C (a typical value for SODIS).[22,36,37] Spectroradiometry and chemical actinometry were undertaken according to procedures described in Text S4 of the Supporting Information.

Competition Kinetics Measurements. The second-order rate constant for reaction of O(^{3}P) with *t*BuOH was measured using competition kinetics,[38] with eq 3 as the reference reaction and O(^{3}P) produced by FAC photolysis under simulated sunlight (see Text S5 of the Supporting Information).

RESULTS AND DISCUSSION

Enhancement of Oocyst Inactivation during FAC Photolysis. Figure 1a compares log-scale measurements of *C. parvum* oocyst infectivity, log(N/N_0) (where N is the number of infective oocysts per milliliter), in 10 mM phosphate buffer (pH 8.0, 25 °C) during exposure to FAC alone (FAC only), simulated sunlight alone (Light only), and FAC and simulated sunlight (FAC+light). Accompanying FAC measurements are depicted in Figure 1b. As shown in Figure 1a, treatment with FAC alone yielded no oocyst inactivation, even at the highest FAC exposures used [$CT_{FAC} = \int_0^t [FAC]\, dt$ = 832 (mg min) L^{-1} (Figure S3 of the Supporting Information)], consistent with prior observations.[10,12,13] Exposure to simulated sunlight alone yielded gradual oocyst inactivation after irradiation for ~40 min (corresponding to Fluence$_{tot,\lambda=290-400\ nm}$ ~ 9 × 10^4 J m^{-2}), also consistent with previous findings.[22,23,25] However, the *combination* of FAC and simulated sunlight dramatically accelerated

dx.doi.org/10.1021/ez500270u | Environ. Sci. Technol. Lett. 2014, 1, 453−458

oocyst inactivation compared to FAC or light alone, yielding 2 log inactivation after irradiation for ~55 min, even though the CT_{FAC} values to which oocysts were exposed under these conditions were substantially lower than for FAC alone (Figure S3 of the Supporting Information), because of the continuous decrease in FAC concentration. These effects are presumably due to the production of reactive oxygen species (ROS) [i.e., HO• and $O(^3P)$], as well as O_3, during FAC photolysis, as reported for *B. subtilis* spores.[19]

In an additional set of experiments designed to explore the possibility of harnessing this chemistry to augment point-of-use SODIS processes, oocysts in pH 8, phosphate buffered Tolt River water were exposed to FAC alone and to natural sunlight in the presence and absence of FAC. As shown in Figure 1c and Figure S3 of the Supporting Information, exposure to FAC alone yielded no oocyst inactivation up to CT_{FAC} = 459 (mg min) L^{-1}. Light only conditions yielded oocyst inactivation at a rate higher than that in simulated sunlight experiments, which could be due to thermal effects,[35,39,40] and/or DOM-photosensitized production of reactive species (e.g., HO• or singlet oxygen)[41] in the Tolt River water. As observed for simulated sunlight, the combination of FAC with natural sunlight dramatically enhanced oocyst inactivation compared to FAC or natural sunlight alone, yielding 2 log inactivation after irradiation for ~45 min (Figure 1c). However, the rate of FAC decay under FAC+light conditions in natural sunlight experiments was ~2-fold faster than in simulated sunlight experiments, necessitating FAC redosing after irradiation for 30 min (Figure 1d). This is likely due to direct FAC consumption by DOM and/or radical chain FAC decomposition initiated by reactions of HO• with DOM.[42,43] Despite the need to redose FAC, the CT_{FAC} levels to which oocysts were exposed during FAC photolysis remained substantially lower than for FAC alone (Figure S3 of the Supporting Information).

In contrast with simulated sunlight experiments, a lag in oocyst inactivation was observed under FAC+light conditions in natural sunlight experiments up to ~10 min of irradiation, after which oocysts were inactivated more rapidly than in the simulated sunlight experiments (Figure 1a,c). Some interexperiment variation in oocyst inactivation kinetics is expected due to inherent variability in oocysts from different stocks or of differing ages.[12] However, the biphasic kinetics of oocyst inactivation in natural sunlight experiments can likely be attributed in part to initial suppression of O_3 and/or ROS levels by certain constituents of the Tolt River water DOM (in analogy with the rapid consumption of O_3 by DOM during the "initial phase" of conventional ozonation processes),[44] followed by a gradual buildup of oxidants in solution. The elevated rate of post-lag-phase oocyst inactivation is consistent with the higher temperature of natural sunlight experiments (noting the positive temperature dependence of oocyst inactivation by O_3 and other disinfectants),[12,26] though DOM-photosensitized reactive species production may also have played a role.

Role of Secondary Oxidants Produced during FAC Photolysis. The roles of HO•, $O(^3P)$, and O_3 in driving oocyst inactivation during FAC photolysis were further investigated by exposing oocysts to FAC and simulated sunlight in (a) the absence of O_2 and (b) the presence of 50 mM *t*BuOH. Deoxygenation precludes the formation of O_3 through eq 3, while leaving HO• and $O(^3P)$ production from eqs 1 and 2 undisturbed. In contrast, *t*BuOH directly scavenges HO• and $O(^3P)$ via eqs 4 and 5[41,45]

$$HO^\bullet + t\text{BuOH} \rightarrow H_2O + \text{products} \qquad k_4 = 6.0 \times 10^8\ M^{-1}\ s^{-1}\ ^{45} \tag{4}$$

$$O(^3P) + t\text{BuOH} \rightarrow HO^\bullet + \text{products} \qquad k_5 = (3.8 \pm 0.1) \times 10^7\ M^{-1}\ s^{-1}\ ^{\text{present work}} \tag{5}$$

where HO• produced by eq 5 is consumed in eq 4, and scavenging of $O(^3P)$ also suppresses O_3 formation from eq 3. An overview of how these effects may influence oocyst inactivation during FAC photolysis is provided in Scheme S1 of the Supporting Information.

As shown in panels a and b of Figure 2, deoxygenation resulted in complete suppression of enhancements in oocyst inactivation during FAC photolysis (i.e., inactivation kinetics for FAC+light w/o O_2 were no different from those for Light only conditions), even though FAC decay kinetics were the same as in the presence of O_2. This indicates that O_3 formation through eq 3 is required to achieve oocyst inactivation during FAC photolysis and also shows that the levels of HO• and $O(^3P)$ generated through eqs 1 and 2 under these conditions are insufficient to yield oocyst inactivation on their own.

As shown in Figure 2c, enhancements in oocyst inactivation during FAC photolysis were also substantially diminished in the presence of 50 mM *t*BuOH. For example, after irradiation for 60 min, the level of oocyst inactivation in the absence of *t*BuOH was 1.4 log higher under FAC+light conditions than for light alone, while in the *presence* of 50 mM *t*BuOH, the level of oocyst inactivation was only 0.3 log higher under FAC+light conditions than for light alone, an 80% reduction in enhancement effects. FAC decay kinetics were unaffected by *t*BuOH (Figure 2d).

Much of the effect of *t*BuOH on oocyst inactivation appears to arise from $O(^3P)$ scavenging (eq 5) and consequent inhibition of O_3 formation from eq 3. On the basis of measurements undertaken in this work (see Text S5 of the Supporting Information), the magnitude of k_5 was determined to be $(3.8 \pm 0.1) \times 10^7\ M^{-1}\ s^{-1}$ at 25 °C. This is significantly higher than a previous estimate of k_5 based on gas-phase kinetics for reaction of $O(^3P)$ with neopentane ($\sim 2.5 \times 10^6\ M^{-1}\ s^{-1}$)[46] but is consistent with measured aqueous-phase rate constants for reactions of $O(^3P)$ with ethanol ($8.8 \times 10^7\ M^{-1}\ s^{-1}$) and 2-propanol ($1.8 \times 10^8\ M^{-1}\ s^{-1}$).[47] Using the values of $k_5 = 3.8 \times 10^7\ M^{-1}\ s^{-1}$ and $k_3 = 4.0 \times 10^9\ M^{-1}\ s^{-1}$, first-order rate constants of $k_5[t\text{BuOH}] = 1.9 \times 10^6\ s^{-1}$ and $k_3[O_2] = 1.1 \times 10^6\ s^{-1}$ were calculated for the *t*BuOH concentration of 5.0×10^{-2} M and the O_2 concentration of 2.8×10^{-4} M, respectively, present in these experiments. Under these conditions, *t*BuOH would have scavenged ~63% of available $O(^3P)$, resulting in an equivalent drop in O_3 production from eq 3. This could account for most of the ~80% drop in the enhancement of oocyst inactivation observed in the presence of *t*BuOH (Figure 2c).

The remainder of the drop in oocyst inactivation caused by *t*BuOH could result from HO• scavenging (eq 4). As noted above, HO• does not directly inactivate oocysts during FAC photolysis. However, it may contribute indirectly to inactivation by enhancing the sensitivity of oocysts to O_3. Such effects have been observed for *B. subtilis* spores, which are inactivated significantly faster by O_3 when also exposed to HO•, apparently due to HO•-driven degradation of O_3-recalcitrant constituents within the spore coat.[48,49] Thus, by scavenging HO•, *t*BuOH may protect oocysts from damage that would otherwise render them more susceptible to inactivation by O_3.

dx.doi.org/10.1021/ez500270u | *Environ. Sci. Technol. Lett.* 2014, 1, 453−458

Taken together, these observations suggest that the enhancements in oocyst inactivation during FAC photolysis are driven primarily by O_3, with $HO^{\bullet}$ possibly playing a complementary role by enhancing the sensitivity of oocysts to O_3. Research to confirm the mechanisms of oocyst inactivation during FAC photolysis is ongoing.

Practical Implications. The combination of chlorination with sunlight could provide an effective, simple, low-cost means of improving *Cryptosporidium* oocyst inactivation during water treatment. The results shown in Figure 1c demonstrate that it should be possible to implement FAC photolysis for point-of-use drinking water disinfection simply by employing chlorine in conventional SODIS procedures. Such an approach could be particularly well suited for use in developing societies, as both chlorination and SODIS are widely accessible across much of the developing world.[35,50] However, the data shown in Figure 1 also illustrate that process efficiency will depend on the presence of DOM and other oxidant scavengers in the treated water, with relatively large FAC doses required during treatment of waters with more than a few milligrams per liter of DOC. Thus, FAC photolysis would likely be most appropriate for treatment of relatively low-DOC waters. Work is currently underway to systematically examine the influence of DOC concentrations and other water quality parameters (e.g., pH, temperature, and alkalinity) on pathogen inactivation, as well as on disinfection byproduct formation, during FAC photolysis.

The findings reported here suggest that there could also be opportunities for employing FAC photolysis in wastewater treatment (e.g., in engineered wetlands), though only if NH_3 and DOC levels are low enough for maintenance of residual FAC. Furthermore, it should be noted that this work could have important implications for outdoor swimming pools, which are frequently disinfected with FAC or other chlorine-containing products, and for which contamination with *Cryptosporidium* oocysts represents a significant public health risk.[51−53]

■ ASSOCIATED CONTENT

Supporting Information

Narratives and graphics addressing Tolt River water samples, oocyst infectivity assays, disinfection procedures, kinetics measurements, and photochemical measurements. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Address: 305 More Hall, Box 352700, Seattle, WA 98195-2700. E-mail: doddm@uw.edu. Phone: (206) 685-7583. Fax: (206) 543-1543.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by National Science Foundation Grant CBET-1236303. We thank Nicola Beck, Christine Fagnant, and Collin White for technical assistance and Jim Nilson for assistance obtaining Tolt River water samples. Three anonymous reviewers are acknowledged for their helpful comments.

■ REFERENCES

(1) Rose, J. B.; Huffman, D. E.; Gennaccaro, A. Risk and control of waterborne cryptosporidiosis. *FEMS Microbiol. Rev.* **2002**, *26*, 113−123.

(2) *Risk Assessment of Cryptosporidium in Drinking Water*; World Health Organization: Geneva, 2009.

(3) Chalmers, R. M.; Davies, A. P. Minireview: Clinical cryptosporidiosis. *Exp. Parasitol.* **2010**, *124*, 138−146.

(4) Xiao, L. Molecular epidemiology of cryptosporidiosis: An update. *Exp. Parasitol.* **2010**, *124*, 80−89.

(5) Jiang, J.; Alderisio, K. A.; Xiao, L. Distribution of *Cryptosporidium* genotypes in storm event water samples from three watersheds in New York. *Appl. Environ. Microbiol.* **2005**, *71*, 4446−4454.

(6) Xiao, L.; Singh, A.; Limor, J.; Graczyk, T. K.; Gradus, S.; Lal, A. Molecular characterization of *Cryptosporidium* oocysts in samples of raw surface water and wastewater. *Appl. Environ. Microbiol.* **2001**, *67*, 1097−1101.

(7) Aboytes, R.; Di Giovanni, G. D.; Abrams, F. A.; Rheinecker, C.; McElroy, W.; Shaw, N.; LeChevallier, M. W. Detection of infectious *Cryptosporidium* in filtered drinking water. *J. Am. Water Works Assoc.* **2004**, *96*, 88−98.

(8) Carey, C.; Lee, H.; Trevors, J. Biology, persistence and detection of *Cryptosporidium parvum* and *Cryptosporidium hominis* oocyst. *Water Res.* **2004**, *38*, 818−862.

(9) *Alternative Disinfectants and Oxidants Guidance Manual*; U.S. Environmental Protection Agency: Washington, DC, 1999.

(10) Rennecker, J. L.; Driedger, A. M.; Rubin, S. A.; Marinas, B. J. Synergy in sequential inactivation of *Cryptosporidium parvum* with ozone/free chlorine and ozone/monochloramine. *Water Res.* **2000**, *34*, 4121−4130.

(11) Li, H.; Finch, G. R.; Smith, D. W.; Belosevic, M. Sequential inactivation of *Cryptosporidium parvum* using ozone and chlorine. *Water Res.* **2001**, *35*, 4339−4348.

(12) Corona-Vasquez, B.; Samuelson, A.; Rennecker, J. L.; Marinas, B. J. Inactivation of *Cryptosporidium parvum* oocysts with ozone and free chlorine. *Water Res.* **2002**, *36*, 4053−4063.

(13) Driedger, A. M.; Rennecker, J. L.; Marinas, B. J. Sequential inactivation of *Cryptosporidium parvum* oocysts with ozone and free chlorine. *Water Res.* **2000**, *34*, 3591−3597.

(14) *Technologies and Costs Document for the Final Long Term 2 Enhanced Surface Water Treatment Rule and Final Stage 2 Disinfectants and Disinfection Byproducts Rule*; U.S. Environmental Protection Agency: Washington, DC, 2006.

(15) *Long Term 2 Enhanced Surface Water Treatment Rule Toolbox Guidance Manual*; U.S. Environmental Protection Agency: Washington, DC, 2010.

(16) Buxton, G. V.; Subhani, M. S. Radiation-chemistry and photochemistry of oxychlorine ions. 2. Photodecomposition of aqueous-solutions of hypochlorite ions. *J. Chem. Soc., Faraday Trans. 1* **1972**, *68*, 958−969.

(17) Nowell, L. H.; Hoigne, J. Photolysis of aqueous chlorine at sunlight and ultraviolet wavelengths. 2. Hydroxyl radical production. *Water Res.* **1992**, *26*, 599−605.

(18) Chan, P. Y.; El-Din, M. G.; Bolton, J. R. A solar-driven UV/chlorine advanced oxidation process. *Water Res.* **2012**, *46*, 5672−5682.

(19) Forsyth, J. E.; Zhou, P.; Mao, Q.; Asato, S. S.; Meschke, J. S.; Dodd, M. C. Enhanced inactivation of *Bacillus subtilis* spores during solar photolysis of free available chlorine. *Environ. Sci. Technol.* **2013**, *47*, 12976−12984.

(20) Klaening, U. K.; Sehested, K.; Wolff, T. Ozone formation in laser flash photolysis of oxoacids and oxoanions of chlorine and bromine. *J. Chem. Soc., Faraday Trans. 1* **1984**, *80*, 2969−2979.

(21) Yu, X. Y. Critical evaluation of rate constants and equilibrium constants of hydrogen peroxide photolysis in acidic aqueous solutions containing chloride ions. *J. Phys. Chem. Ref. Data* **2004**, *33*, 747−763.

(22) McGuigan, K.; Méndez-Hermida, F.; Castro-Hermida, J.; Ares-Mazás, E.; Kehoe, S.; Boyle, M.; Sichel, C.; Fernández-Ibáñez, P.; Meyer, B.; Ramalingham, S. Batch solar disinfection inactivates oocysts of *Cryptosporidium parvum* and cysts of *Giardia muris* in drinking water. *J. Appl. Microbiol.* **2006**, *101*, 453−463.

(23) Connelly, S. J.; Wolyniak, E. A.; Williamson, C. E.; Jellison, K. L. Artificial UV-B and solar radiation reduce in vitro infectivity of the human pathogen *Cryptosporidium parvum*. *Environ. Sci. Technol.* **2007**, *41*, 7101−7106.

(24) Cho, M.; Yoon, J. Measurement of OH radical CT for inactivating *Cryptosporidium parvum* using photo/ferrioxalate and photo/TiO_2 systems. *J. Appl. Microbiol.* **2008**, *104*, 759−766.
(25) King, B. J.; Hoefel, D.; Daminato, D. P.; Fanok, S.; Monis, P. T. Solar UV reduces *Cryptosporidium parvum* oocyst infectivity in environmental waters. *J. Appl. Microbiol.* **2008**, *104*, 1311−1323.
(26) Driedger, A.; Staub, E.; Pinkernell, U.; Marinas, B.; Koster, W.; Von Gunten, U. Inactivation of *Bacillus subtilis* spores and formation of bromate during ozonation. *Water Res.* **2001**, *35*, 2950−2960.
(27) Larson, M. A.; Marinas, B. J. Inactivation of *Bacillus subtilis* spores with ozone and monochloramine. *Water Res.* **2003**, *37*, 833−844.
(28) Cho, M.; Kim, J. H.; Yoon, J. Investigating synergism during sequential inactivation of *Bacillus subtilis* spores with several disinfectants. *Water Res.* **2006**, *40*, 2911−2920.
(29) Kumar, K.; Day, R. A.; Margerum, D. W. Atom-transfer redox kinetics: General-acid-assisted oxidation of iodide by chloramines and hypochlorite. *Inorg. Chem.* **1986**, *25*, 4344−4350.
(30) APHA. *Standard Methods for the Examination of Water and Wastewater*, 21st ed.; APHA, AWWA, WPCF: Washington, DC, 2005.
(31) King, B. J.; Keegan, A. R.; Phillips, R.; Fanok, S.; Monis, P. T. Dissection of the hierarchy and synergism of the bile derived signal on *Cryptosporidium parvum* excystation and infectivity. *Parasitology* **2012**, *139*, 1533−1546.
(32) Sifuentes, L. Y.; Di Giovanni, G. D. Aged HCT-8 cell monolayers support *Cryptosporidium parvum* infection. *Appl. Environ. Microbiol.* **2007**, *73*, 7548−7551.
(33) Johnson, A. M.; Di Giovanni, G. D.; Rochelle, P. A. Comparison of assays for sensitive and reproducible detection of cell culture-infectious *Cryptosporidium parvum* and *Cryptosporidium hominis* in drinking water. *Appl. Environ. Microbiol.* **2012**, *78*, 156−162.
(34) APHA. *Standard Methods for the Examination of Water and Wastewater*, 22nd ed.; APHA, AWWA, WPCF: Washington, DC, 2012.
(35) McGuigan, K. G.; Conroy, R. M.; Mosler, H. J.; du Preez, M.; Ubomba-Jaswa, E.; Fernandez-Ibanez, P. Solar water disinfection (SODIS): A review from bench-top to roof-top. *J. Hazard. Mater.* **2012**, *235*, 29−46.
(36) Dejung, S.; Fuentes, I.; Almanza, G.; Jarro, R.; Navarro, L.; Arias, G.; Urquieta, E.; Torrico, A.; Fenandez, W.; Iriarte, M. Effect of solar water disinfection (SODIS) on model microorganisms under improved and field SODIS conditions. *J. Water Supply: Res. Technol.−Aqua* **2007**, *56*, 245−256.
(37) Boyle, M.; Sichel, C.; Fernández-Ibáñez, P.; Arias-Quiroz, G.; Iriarte-Puna, M.; Mercado, A.; Ubomba-Jaswa, E.; McGuigan, K. Bactericidal effect of solar water disinfection under real sunlight conditions. *Appl. Environ. Microbiol.* **2008**, *74*, 2997−3001.
(38) Muñoz, F.; von Sonntag, C. Determination of fast ozone reactions in aqueous solution by competition kinetics. *J. Chem. Soc., Perkin Trans. 2* **2000**, *2*, 661−664.
(39) Wegelin, M.; Canonica, S.; Mechsner, K.; Fleischmann, T.; Pesaro, F.; Metzler, A. Solar water disinfection: Scope of the process and analysis of radiation experiments. *J. Water Supply: Res. Technol.−Aqua* **1994**, *43*, 154−169.
(40) Gomez-Couso, H.; Fontan-Sainz, M.; Ares-Mazas, E. Thermal contribution to the inactivation of *Cryptosporidium* in plastic bottles during solar water disinfection procedures. *Am. J. Trop. Med. Hyg.* **2010**, *82*, 35−39.
(41) Foote, C. S., Selverstone Valentine, J., Greenberg, A., Liebman, J. F., Eds. *Active Oxygen in Chemistry*; Blackie Academic and Professional: New York, 1995; Vol. *2*.
(42) Oliver, B. G.; Carey, J. H. Photochemical production of chlorinated organics in aqueous solutions containing chlorine. *Environ. Sci. Technol.* **1977**, *11*, 893−895.
(43) Jin, J.; El-Din, M. G.; Bolton, J. R. Assessment of the UV/chlorine process as an advanced oxidation process. *Water Res.* **2011**, *45*, 1890−1896.
(44) Buffle, M. O.; von Gunten, U. Phenols and amine induced $HO^{\bullet}$ generation during the initial phase of natural water ozonation. *Environ. Sci. Technol.* **2006**, *40*, 3057−3063.
(45) Buxton, G. V.; Greenstock, W. P.; Helman, W. P.; Ross, A. B. Critical review of rate constants for reactions of hydrated electrons, hydrogen atoms, and hydroxyl radicals ($^{\bullet}OH/O^{\bullet-}$) in aqueous solution. *J. Phys. Chem. Ref. Data* **1988**, *17*, 513−886.
(46) Reisz, E.; Schmidt, W.; Schuchmann, H.-P.; von Sonntag, C. Photolysis of ozone in aqueous solutions in the presence of tertiary butanol. *Environ. Sci. Technol.* **2003**, *37*, 1941−1948.
(47) Amichai, O.; Treinin, A. Chemical reactivity of $O(^3P)$ atoms in aqueous solution. *Chem. Phys. Lett.* **1969**, *3*, 611−613.
(48) Cho, M.; Chung, H. M.; Yoon, J. Disinfection of water containing natural organic matter by using ozone-initiated radical reactions. *Appl. Environ. Microbiol.* **2003**, *69*, 2284−2291.
(49) Cho, M.; Yoon, J. Enhanced bactericidal effect of O_3/H_2O_2 followed by Cl_2. *Ozone: Sci. Eng.* **2006**, *28*, 335−340.
(50) The Safe Water System (http://www.cdc.gov/safewater/index.html); Centers for Disease Control and Prevention: Atlanta, 2013.
(51) Shields, J. M.; Hill, V. R.; Arrowood, M. J.; Beach, M. J. Inactivation of *Cryptosporidium parvum* under chlorinated recreational water conditions. *J. Water Health* **2008**, *6*, 513−520.
(52) Shields, J. M.; Gleim, E. R.; Beach, M. J. Prevalence of *Cryptosporidium* spp. and *Giardia intestinalis* in swimming pools, Atlanta, Georgia. *Emerging Infect. Dis.* **2008**, *14*, 948−950.
(53) Waldron, L. S.; Ferrari, B. C.; Cheung-Kwok-Sang, C.; Beggs, P. J.; Stephens, N.; Power, M. L. Molecular epidemiology and spatial distribution of a waterborne Cryptosporidiosis outbreak in Australia. *Appl. Environ. Microbiol.* **2011**, *77*, 7766−7771.