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Inactivation of *E. coli*, *B. subtilis* spores, and MS2, T4, and T7 phage using UV/H_2O_2 advanced oxidation

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Abstract

The goal of this study was to evaluate the potential of an advanced oxidation process (AOP) for microbiocidal and virucidal inactivation. The viruses chosen for this study were bacteriophage MS2, T4, and T7. In addition, *Bacillus subtilis* spores and *Escherichia coli* were studied. By using H_2O_2 in the presence of filtered ultraviolet (UV) irradiation (UV/H_2O_2) to generate wavelengths above 295 nm, the direct UV photolysis disinfection mechanism was minimized, while disinfection by H_2O_2 was also negligible. Virus T4 and *E. coli* in phosphate buffered saline (PBS) were sensitive to >295 nm filtered UV irradiation (without H_2O_2), while MS2 was very resistant. Addition of H_2O_2 at 25 mg/l in the presence of filtered UV irradiation over a 15 min reaction time did not result in any additional disinfection of virus T4, while an additional one log inactivation for T7 and 2.5 logs for MS2 were obtained. With *E. coli*, only a slight additional effect was observed when H_2O_2 was added. *B. subtilis* spores did not show any inactivation at any of the conditions used in this study. The OH radical exposure (CT value) was calculated to present the relationship between the hydroxyl radical dose and microbial inactivation.

Keywords: AOP; Hydroxyl radical; Oxidation; Ultraviolet; Disinfection; CT

1. Introduction

Ultraviolet light (UV) has been shown to be very effective for the disinfection of drinking water and wastewater [1], due to its absorption by the DNA or RNA of microorganisms. In addition to disinfection, direct photolysis for removal of contaminants was shown to be effective when the absorption spectrum of the pollutant overlaps the emission spectrum of the UV lamp, and the quantum yield of the photochemical process is reasonably large. The application of UV in combination with hydrogen peroxide (UV/H_2O_2) brings about enhanced oxidative degradation of pollutants by the generation of highly reactive hydroxyl radical ($^{\bullet}OH$). When UV light is absorbed directly by H_2O_2, hydroxyl radicals are generated by photolysis of the peroxidic bond (Eq. (1)). The most efficient hydroxyl radical yields are obtained when short wave UV wavelengths (200–280 nm) are used.

$$H_2O_2 + h\nu \rightarrow 2^{\bullet}OH \quad (1)$$

The theoretical quantum yield for generating hydroxyl radicals using UV/H_2O_2 process is 1.0 [2]. A combined UV/H_2O_2 process can ideally be used for means of both breaking down pollutants and inactivating microorganisms. Numerous studies report that the UV/H_2O_2 advanced oxidation process (AOP) is effective for the destruction of various pollutants such as pesticides and pharmaceuticals [3–7], however there are very few available reports on inactivation of microorganisms via OH radical oxidation.

In general, two primary mechanisms control the oxidant disinfection efficiency by hydroxyl radicals: (1) oxidation and disruption of the cell wall and membrane with resulting disintegration of the cell (oxidation ability is due in part to its standard reduction potential (2.70 V for hydroxyl radicals)), and (2) diffusion of the disinfectant into the cell or particle where it may inactivate enzymes, damage intracellular components, interfere with protein synthesis, etc. [8]. Diffusion of the disinfecting species into the cells is a function of the charge, molecular weight, and half-life of the disinfectant. Hydroxyl radical reacts with most biological molecules at diffusion-controlled rates. Therefore, disinfection by hydroxyl radicals may be limited by mass transfer through the cell wall or cell membrane [9]. Titanium dioxide-mediated poliovirus inactivation, although slow, was reported to

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be four times faster than coliform bacteria inactivation, which correlates with the diffusion-controlled oxidation that may be occurring. The lower surface to volume ratio of the viruses may provide greater rates of hydroxyl radical reaction with intracellular biological molecules compared with the larger bacterial cells. The relatively slow diffusion of hydroxyl radicals into viruses, and particularly bacterial cells, may be the cause of its low disinfection rate, and may limit its use as a disinfectant [9].

The goal of this study was to demonstrate and contrast the potential of an AOP-UV/H_2O_2-for microbiocidal and virucidal inactivation. To minimize the impact of direct UV photolysis in the AOP system and isolate the effect of the $^{\bullet}$OH radical, UV radiation between 200 and 295 nm was filtered out. The viruses chosen for this study were bacteriophage MS2 (F+ specific, single-stranded RNA, similar in size and shape to human enteric viruses, used for UV reactor validation), bacteriophage T4 (one of the largest of the bacterial viruses, double-stranded DNA), and T7 (biosensor for solar UV, double-stranded DNA). In addition, *Bacillus subtilis* spores (used in UV reactor validation, possible surrogate for protozoa with ozone) and *Escherichia coli* (representative for traditional indicator bacteria in water) were studied.

2. Materials and methods

2.1. Preparation and enumeration of microorganisms

Bacteria: (a) *B. subtilis* spores (ATCC 6633) were produced by a liquid cultivation technique and obtained freeze-dried [10]. Working solution was prepared by resuspending the spores in water. Spores were enumerated by pour plate technique with PC-Agar (Oxoid CM 325), at 37 °C for two days, as colony forming units (CFU) per ml [10]. (b) *E. coli* B (ATCC 11303) was cultivated using the recommended tryptone based broth in incubator at 37 °C shaking for 4 h till the concentration was 10^8 cells/ml. Enumeration as CFU was performed by spread plate with Mcconkey agar and tryptone based agar.

Bacteriophages: (a) MS2 (ATCC 15977-B1) was propagated and enumerated according to the International standard ISO 10705-1 [11], with host *E. coli* bacteria C3000 (ATCC 15597). The sample containing MS2 was diluted as desired and 0.1 ml was added into a test tube with 4 ml of soft agar and 1 ml of *E. coli* host at 10^8 cells/ml, and kept in water bath at 43–45 °C. The mixture was immediately poured on TYGA agar plate and allowed to solidify. The mixture was then incubated upside-down at 37 °C for 18 h, after which the MS2 plaques were counted. (b) Stocks of T4 and T7 were received from Clancy Environmental Consultants, Inc. (St Albans, VT). Phages were diluted in PBS (Fisher scientific, USA) for the experiments to yield 10^6 counts/ml, and propagated and enumerated according to Adams [12]. Briefly, *E. coli* B host (ATCC 11303) was grown in tryptic soy and sodium chloride broth in incubator at 37 °C shaking for 4 h till the concentration was 10^8 cells/ml. 0.1 ml of the appropriate phage dilution was added into the tube containing tryptone based top agar tempered at 45 °C and *E. coli* host. The solution was mixed and poured on tryptone based bottom agar in Petri dish. Samples were plated in triplicate, allowed to solidify, and than incubated upside-down at 37 °C for 5 and 10 h for T7 and T4, respectively. For all bacteriophages, negative controls (PBS containing host bacteria and top agar only), and positive controls were also plated. After incubation, plates with 20–200 plaques (PFU) were counted.

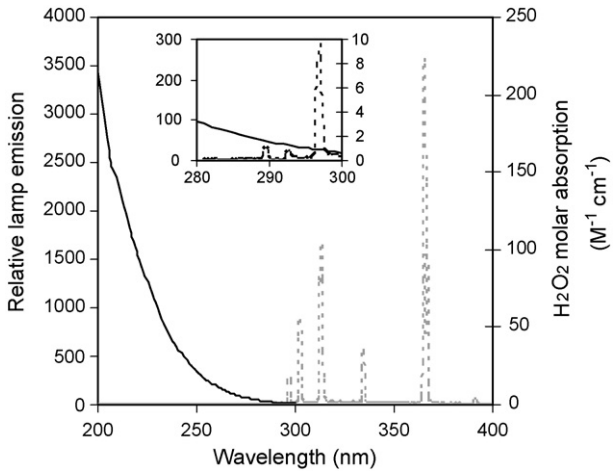

Fig. 1. Emission spectrum of the MP lamp after passing through the 295 nm long-pass filter and molar absorption of H_2O_2. The insert represents the overlap between MP lamp emission and molar absorbance of H_2O_2 between 280 and 300 nm.

2.2. Experimental setup

Photolysis experiments were carried out with a medium pressure (MP) mercury lamp (Hanovia Co., Union, NJ) in bench scale UV collimated beam apparatus. Experiments were conducted with a 295 nm rectangular long-pass filter (2.5 mm width; Schott, USA) placed in the light path. The spectrum of the filtered lamp is shown in Fig. 1. A 100 ml sample was spiked with either *E. coli*, *B. subtilis* spores, T4, T7, or MS2 at initial concentration of 10^6 CFU/ml or PFU/ml, and irradiated with gentle stirring in a 70 × 50 mm crystallization dish (34.2 cm^2 surface area, solution depth approximately 3.3 cm) open to the atmosphere. Samples were collected over a minimum of seven targeted contact times (0, 2.5, 5, 7.5, 10, 12.5, and 15 min, respectively). Total UV incident irradiance (at wavelength range between 295 and 400 nm) was 0.78 mW/cm^2 measured with a USB4000 spectrometer (Ocean Optics Inc. Dunedin, FL). Addition of H_2O_2 reduced the average dose (fluence) delivered to the microorganisms by less than 0.5%.

Control experiments were designed to examine if the bactericidal source for inactivation resulted from H_2O_2, the UV source, or the combined UV/H_2O_2 process. One of the challenges in this study was to separate the outcome of UV/H_2O_2 OH radical microbial disinfection from UV. It was determined that using H_2O_2 in the presence of UV irradiation above 295 nm would result in minimal direct UV disinfection. Thus, the conditions tested in this study were: polychromatic UV source (cutoff UV < 295 nm), H_2O_2 (0–25 mg/l; (30% w/w, Fluka Germany), and the combined UV/H_2O_2 (0–25 mg/l, UV > 295 nm) on inactivation of test organisms in buffered (PBS) and surface waters (SW).

For H_2O_2 control experiments samples were collected at a minimum of three targeted contact times (0, 30, and 60 min, respectively), and residual H_2O_2 was measured by the KI-thiosulfate method outlined in Kolthoff and Sandell [13]. Catalase was added in order to destroy residual H_2O_2 prior to microbial/chemical analysis. The UV/H_2O_2 experiments were designed similarly to the UV experiments but with the addition of H_2O_2 to the PBS or SW suspension at a beginning target concentration of 0, 10, and 25 mg/l H_2O_2, respectively, and 10^6 CFU/ml or PFU/ml of microorganisms.

2.3. Hydroxyl radicals concentration

Hydroxyl radical concentration in aqueous medium was indirectly determined by the rate of consumption of a probe compound, *para*-chlorobenzoic acid (*p*CBA) [14]. The steady state concentration of the hydroxyl radicals was estimated according to Eq. (2).

$$-\frac{\mathrm{d}\left[p\mathrm{CBA}\right]}{\mathrm{d}t} = k_{\mathrm{OH}p\mathrm{CBA}}\left[p\mathrm{CBA}\right]\left[^{\bullet}\mathrm{OH}\right]_{\mathrm{ss}} \quad (2)$$

where, $k_{\mathrm{OH}p\mathrm{CBA}}$is the rate constant of *p*CBA with hydroxyl radicals, $5 \times 10^9\ \mathrm{M^{-1}s^{-1}}$ (NDRL), at ambient temperature and pressure, $[^{\bullet}\mathrm{OH}]_{\mathrm{ss}}$ is the steady state concentration of hydroxyl radicals.

2.4. Analytical methods

*p*CBA concentration was determined by C-18 (7.5 × 150 mm) reverse phase chromatography using a Varian Prostar HPLC (Varian, INC., Palo Alto, CA) equipped with a photodiode array detector. Isocratic elution was used with a mobile phase of 50:50 ACN:H_2O at pH 2, at flow rate of 1 ml/min, 100 μl injection, and absorbance detection at 240 nm. The retention time for *p*CBA was 4.1 min. The system was calibrated using aqueous dilution of standards dissolved in methanol.

Total organic carbon (TOC) was measured by a Tekmar Dohrmann Apollo 9000 total carbon analyzer in accordance with Standard Method 5310A [15]. Turbidity was determined using Hach 2100N turbidimeter. pH of the solution was determined by using a Thermo Orion 920A pH meter. Alkalinity and total hardness were measured according to the standard methods 2320 and 2340, respectively [15]. Anion concentration was measured with DX-120 Dionex ion chromatograph equipped with an AS 14A column, eluent 8 mM Na_2CO_3, and 1 mM $NaHCO_3$.

2.5. Surface water

Natural water was collected from the intake to the Wilson water treatment plant in Durham NC, USA. Samples were taken 10–20 cm below the water surface, and collected in 4 liter amber glass bottles. Samples were filtered under vacuum the same day of sampling using washed Millipore glass fiber filters of 0.45 μm pore size. The filtrate was refrigerated in amber glass bottles in the dark at 4 °C. Experiments were conducted within a week of sampling. The water quality of the SW water is presented in Table 1.

2.6. Data presentation

Mean concentration of microorganisms spiked in suspension without UV exposure was taken as the initial concentration, N_0. Mean concentration of microorganisms spiked in suspension with UV exposure was taken as concentration per time (N_d). The $\log_{10}$ transformation for N_0/N_d was plotted as a function of time or $^{\bullet}$OH exposure. The linear curve was described by the following equation where k is the rate constant:

$$\log_{10}\left(\frac{N_0}{N_d}\right) = k \times t \quad (3)$$

3. Results and discussion

3.1. Effect of H_2O_2

The goal of this study was to obtain data on the inactivation of microorganisms with $^{\bullet}$OH generated via UV/H_2O_2 AOP in water. At first, control experiments were conducted to test the effect of H_2O_2 alone on inactivation of the targeted microorganisms. Results showed no inactivation of any microorganisms at a H_2O_2 dose of up to 25 mg/l at 60 min contact time. These results are in good agreement with previously published data. Sommer et al. [16] showed that H_2O_2 at a concentration of 50 mg/l with 3 h contact did not have any virucidal activity and showed weak bactericidal activity. Cho et al. [17] showed inactivation of *E. coli* at higher H_2O_2 concentration, and longer contact times than used in this research. Bianchini et al. [18] did not find any influence of H_2O_2 on coliform inactivation at a dose of 20 mg/l H_2O_2 and contact time of 30 min.

3.2. Effect of filtered UV (λ > 295 nm)

The next step was to test the effect of filtered UV alone (λ > 295 nm) on microorganisms using a polychromatic MP UV source and a 295 nm long-pass filter (See Fig. 1). Fig. 2

Table 1
SW quality obtained from Durham water treatment plant

pH	Alk	TH	TOC (mg/l C)	Conductivity (μs)	Turbidity (NTU)	Cl^- (mg/l)	NO_3^- (mg/l)	PO_4^{3-} (mg/l)	SO_4^{2-} (mg/l)
	mg/l as $CaCO_3$								
7.0	21.0	14.5	10.4	90.0	0.3	7.7	0.7	0.1	5.7

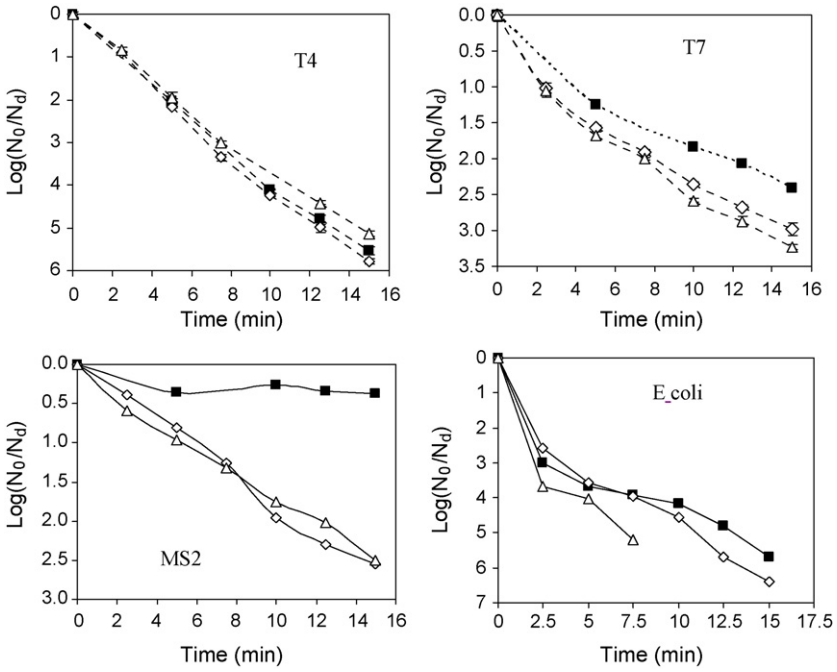

Fig. 2. Time based response curve for T4, T7, *E. coli*, and MS2 by UV/H_2O_2 in PBS ($\lambda > 295$ nm, ■ 0 mg/l H_2O_2, ◊ 10 mg/l H_2O_2, and △ 25 mg/l H_2O_2).

illustrates the time based inactivation of *E. coli*, T4, T7, and MS2 by UV ($\lambda > 295$ nm) and UV/H_2O_2 ($\lambda > 295$ nm, 10, and 25 mg/l H_2O_2) in PBS. The time based inactivation of *E. coli*, T4, T7, and MS2 by filtered UV alone in PBS is displayed as the 0 mg/L H_2O_2 plots. *E. coli* inactivation by filtered UV light was more rapid (up to four logs at 5 min exposure time) compared to the viruses. The rapid inactivation of *E. coli* was followed by a tailing plateau after 5 min of exposure. T4 virus also reached four logs of inactivation, but required 10 min of exposure, while T7 was more resistant, and achieved only two logs of inactivation at similar time. On the other hand, the virus MS2 was unaffected (resistant) by UV wavelengths above 295 nm.

Filtered UV includes wavelengths in the UVB range (290–320 nm), UVA range (320–400 nm), and in the visible range above 400 nm. UVB radiation is a minor part of the solar spectrum; however it has a disproportional metabolic effect on microorganisms [19]. Two possible mechanisms of microbial inactivation in the UVB wavelength range were proposed: (a) direct photolysis (dimer production), and (b) natural occurring indirect oxidative damage through production of intercellular reactive oxygen species (ROS). ROS include species such as superoxide radical, hydroxyl radical, hydrogen peroxide, peroxyl, and peroxynitrite. These species are mostly short lived and unstable, and can cause lipid per-oxidation, DNA strand breakage, and oxidative damage in cells [20]. Under UVB radiation, the formation of ROS depends on, but is not proportional to, the UVB dose. At high doses of UVB, formation of pyrimidine dimers and other photoproducts that are lethal, result in direct cell death [20]. In this study, the role of ROS production in cells as compared to direct damage by formation of dimers was not evaluated, as the relative cause for cell death due to UVB exposure was determined as a control study, and not the goal of this study.

Photochemical reactions for inactivation are most efficient with wavelengths close to the maximum absorbance (∼260 nm) of pyrimidine (thymine, cytosine, and uracil) and purine (adenine and guanine) nucleobases [21]. Thus, inactivation mechanisms at 260 nm may be different from wavelengths above 295 nm. In the environment, the ozone layer filters out the UVC component of the sunlight, therefore UV irradiation that actually reaches earth is between wavelengths of 295–400 nm [22], similar to the UV wavelengths used this study. Within the solar spectrum, *E. coli* is primarily inactivated by direct absorption of the UVB portion of sunlight by DNA [23]. However, wavelengths between 370–550 nm are responsible for restoring infectivity by inducing light repair mechanisms in bacteria and some viruses through removal of pyrimidine dimers [24]. Because the MP UV system used in this research also emits wavelengths in the visible region (data not shown) it might result in co-occurring repair of dimers formed by UVB wavelengths, although it is not expected to significantly effect the inactivation of the microorganisms due to the short time of exposure.

The sensitivity for inactivation of the viruses studied to filtered UV followed a decreasing order of: T4 > T7 > MS2, while MS2 did not show any inactivation when exposed to wavelengths above 295 nm. T4 and T7 phage have been proposed as biosensors for solar radiation [25], thus it was expected that both T4 and T7 would be sensitive to wavelengths above 295 nm. Similar order of sensitivities was reported by Clancy et al. [26] using a 254 nm lamp, where a two log inactivation resulted from a dose of less than 5, 8, and 40 mJ/cm^2 for T4, T7, and MS2 inactivation, respectively. Templeton et al. [27]

found that at 254 nm, MS2 showed two log inactivation at a dose of 33 mJ/cm^2, while T4 was much more sensitive requiring only a dose of 2.5 mJ/cm^2. Inactivation of viruses at 254 nm is mainly due to damage of nucleic acids, as DNA and RNA have the highest 254 nm absorption coefficient between 200–300 nm as compared to other viral components. However, similarity in the order of the phages sensitivities at 254 nm, and at filtered UV further support the assumption that even for filtered UV, the dimer formation is a major inactivation mechanism of direct photolysis rather than ROS species. DNA based viruses are possibly more sensitive to direct UV compared to RNA viruses [28]. Moreover, dimer repair due to UV exposure is less efficient with uracil dimers (nucleotide base of RNA) compared to thymine dimers (nucleotide base of DNA) [29]. Hence, MS2 which is an RNA virus is more resistant to filtered UV as compared to the *T* series viruses which are DNA viruses.

UV 265 nm was found to be very efficient in microbial inactivation of MS2 resulting in two log inactivation at 30 mJ/cm^2 with higher sensitivity (three-fold) at wavelengths below 220 nm [30], where protein absorption is significant [28]. MS2 inactivation at 293 nm was only about 25 percent as effective as 265 nm, suggesting the lack of effectiveness of wavelengths above 295 nm for MS2 phage.

3.3. UV/H_2O_2 ($\lambda > 295$ nm)

Fig. 2 illustrates that advanced oxidation by UV/H_2O_2 (25 mg/l H_2O_2) did not have any additive effect as compared to UV > 295 nm on the T4 virus up to 15 min of reaction time. The same UV/H_2O_2 conditions resulted in an additional one log inactivation for the T7 virus in PBS with advanced oxidation compared to UV alone. *E. coli* under UV/H_2O_2 (10 mg/l) showed similar inactivation to UV > 295 nm at up to 15 min reaction time. *E. coli* under UV/H_2O_2 (25 mg/l) showed a slight additional effect at 7.5 min of reaction time but it was not possible to obtain data over an extended reaction time because the microorganism enumeration was near the detection limit. An additional two log inactivation of MS2 virus occurred with the combined UV/H_2O_2 (up to 25 mg/l) process as compared to UV > 295 nm. Similar results were reported by Cho et al. [31] who showed that while MS2 phage was inactivated by $^\bullet$OH radical, the same level of $^\bullet$OH radicals hardly played a role in the inactivation of *E. coli.* These results were explained by the differences in the sizes and surface structures of these two microorganisms.

UV/H_2O_2 brings about oxidation by the generation of the highly reactive $^\bullet$OH radical. Hydroxyl radicals are able to kill bacteria and viruses mainly by destroying their cell membrane or walls [9,32]. Inactivation of MS2 is induced by denaturing the protein of the capsid, whose structure is simple and rigid, therefore requiring high oxidation power [31]. Gehringer et al. [33] reported exceptionally high sensitivity of MS2 to radical attack formed by electron beam radiation. The differences in OH radical sensitivity between the phages could not be explained by current literature but may have been overshadowed by the high direct UV >295 nm activity on the T4 and T7 phage.

3.4. Hydroxyl radical exposure (CT value)

The OH radical exposure (CT value) represents the OH radical steady state concentration (C) multiplied by the contact time (t) from the start of the experiment through sampling of the microorganisms from the irradiation dish. The steady state hydroxyl radical CT value was determined in order to better understand the relationship between $^\bullet$OH radicals generated and microbial inactivation. The OH radical steady state concentration was determined via decay data for *p*CBA degradation (data not shown) using Eq. (2) with high linear coefficient ($R^2 > 0.99$ for both 10 and 25 mg/l H_2O_2). H_2O_2 scavenging of $^\bullet$OH and light screening from the microorganisms are all taken into account using *p*CBA to determine the concentration of $^\bullet$OH radicals. It is also important to note that the inactivation of the microorganisms at UV >295 nm was subtracted from the total inactivation by the UV/H_2O_2 process, hence $\log(N_0/N_d)$ represents only the effect of the OH radical generated by the AOP process.

Fig. 3 illustrates the inactivation of T7, *E. coli*, and MS2 by UV/H_2O_2 ($\lambda > 295$ nm, 10, and 25 mg/l H_2O_2) on a CT basis. The OH radical CT for achieving 0.5 log inactivation for T7, *E. coli*, and MS2 is 1.47×10^{-13}, 1.47×10^{-13}, and 1.18×10^{-13} M min, respectively. The OH radical CT for achieving 1.25 logs inactivation for *E. coli* and MS2 are 2.06×10^{-13} and 2.53×10^{-13} M min, respectively, while T7 does not reach 1.25 logs from OH radical disinfection. T4 was not affected by the $^\bullet$OH radicals and is therefore not included in the discussion regarding the CT. Results plotted on CT basis showed that up to 0.75 log reduction, a lower CT is required for T7 as compared to *E. coli,* while above 0.75 log inactivation the trend was reversed. These results might be explained by a sharp inactivation of *E. coli* at low filtered UV doses that tail off (Fig. 2), while T7 followed a steady inactivation curve (Fig. 2). Cho et al. [34] reported a CT value of 1.5×10^{-5} mg min/l for

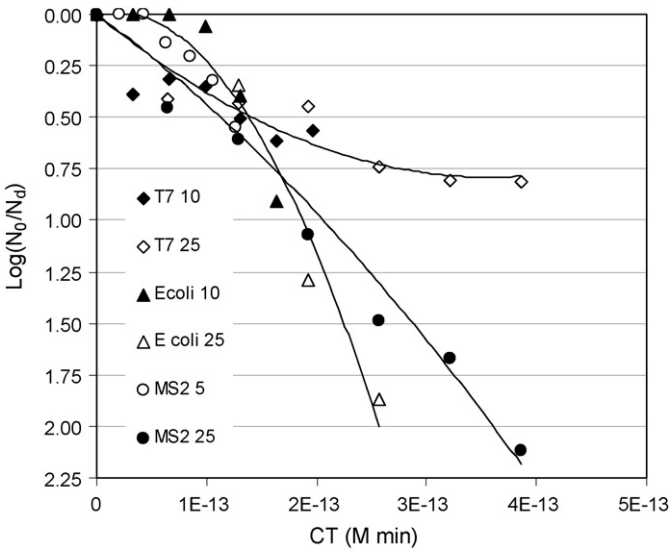

Fig. 3. CT based inactivation of T7, *E. coli*, and MS2 by UV/H_2O_2 ($\lambda > 295$ nm, 10 and 25 mg/l H_2O_2) in PBS. Numbers in the legends refer to H_2O_2 concentration.

two log inactivation of *E. coli*. In the current study, a CT value of approximately 4.5×10^{-9} mg min/l (which corresponds to 2.65×10^{-13} M min) was achieved for the same log inactivation. The differences might be a result of different strains of *E. coli* or the methods used to generate $^{\bullet}$OH radical.

Most of the AOP studies relate inactivation of microorganisms to $^{\bullet}$OH radicals however a few studies discuss formation of other species. Bianchini et al. [18] suggested formation of intermediate free radicals (secondary radicals) during oxidation of wastewater such as hydroxyl radicals and carbon centred radicals. They found that when AOP with UV photolysis is conducted the hydroxyl radical formation is dominant (although other species are formed), and with other treatments carbon centred radicals can be dominant. Kikuchi et al. [35] suggested that possibly superoxide radicals and hydrogen peroxide can diffuse into microorganisms' cell through the membrane, and produce hydroxyl radicals by the Haber–Weiss reaction. UVB induces formation of the superoxide species that can react to form hydrogen peroxide, and if not scavenged can enhance the production of intracellular hydroxyl radical through Fenton or Haber–Weiss reaction [20]. These intracellular ROS species require development and application of methods such as fluorescent probes with combination of flow cytometry or fluorimetry [20], and cannot be measured by *p*CBA method as conducted in this study. Thus, by plotting the experimental data on a CT basis, which considers only the bulk solution concentration of hydroxyl radicals (extracellular), the effect of other radicals or species formed by UV/H_2O_2 or intracellular hydroxyl radicals formed by UVB are inherently ignored.

Hydroxyl radical concentration in a UV/H_2O_2 system is a function of the fraction of light absorbed by the hydrogen peroxide and competition between the microorganisms, scavengers present in the background water matrix, and hydrogen peroxide for hydroxyl radical. In a standard low pressure ($\lambda = 254$ nm) and MP UV ($\lambda = 200$–300 nm) collimated beam apparatus, the concentration of hydroxyl radicals is generally on the order of 10^{-13} to 10^{-12} M in the presence of 2–10 mg/l hydrogen peroxide. The lower OH radical concentration in the experimental system used in this research (10^{-14} to 10^{-13} M) was due to the use of UV at wavelengths >295 nm (i.e. nongermicidal) whereas with nonfiltered LP or MP UV sources, there is a better overlap between lamp emission spectrum and absorption spectrum of hydrogen peroxide, resulting in higher OH radical concentration. Thus, it is expected that lower UV fluence would be required to obtain an equivalent level of OH radical using UVC irradiation. Considering the fact that different mechanisms account for UV and OH radical disinfection, the relative contribution of respective UVC and OH radical based inactivation under engineered water treatment conditions for OH radical generation needs to be assessed in a quantitative manner to determine the importance of OH in inactivation of specific pathogens.

3.5. Inactivation in natural water

Inactivation of MS2 was studied in natural SW obtained from the Durham WTP. The water characteristics are presented in Table 1. Unexpectedly, the inactivation of MS2 in the SW was

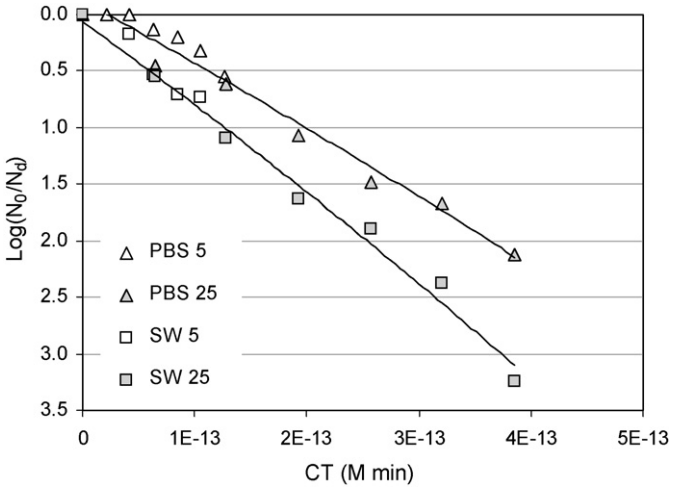

Fig. 4. CT based inactivation of MS2 by UV/H_2O_2 ($\lambda > 295$ nm, 5 and 25 mg/l H_2O_2) in PBS and SW. Numbers in the legends refer to H_2O_2 concentration.

enhanced as compared to buffered waters, as the SW alkalinity and natural organic matter (NOM) are OH radical scavengers, and were expected to decrease the radicals available for disinfection. Alkalinity and NOM concentration in the Durham SW (Table 1) lowered the steady state concentration of OH radicals in the SW to 35–50% below the buffered water. For example, the OH radical concentration at 5 mg/l H_2O_2 was 8.46×10^{-15} M in PBS and 4.26×10^{-15} M in SW, and at 25 mg/l H_2O_2 was 2.57×10^{-14} M in PBS and 1.72×10^{-14} M in SW. Clearly, the scavenging effect of the SW did not result in decreased inactivation of MS2 (Fig. 4). A similar observation was reported by Rincon et al. [36] showing an increase in *E. coli* inactivation in natural waters as compared to deionized water. This phenomenon might be explained by formation of reactive oxidizing species other than OH radicals.

NOM in SW plays an important role in sunlight induced photochemical processes (photosensitizer), not only as a radical scavenger but also as a precursor for ROS [37]. Maness et al. [38] reported that ROS (such as, H_2O_2 and $^{\bullet}O_2^-$, etc.), as well as the OH radical, played a significant role in microorganism inactivation. Davies-Colley et al. [23] suggested that longer wavelengths absorbed by photosensitizers can result in photochemical damage to host binding proteins or phage capsid with F-RNA phage being more sensitive than F-DNA phage. If the NOM sites related to the radical promotion reaction are larger compared to that for the radical–scavenging reaction, then greater OH radical formation can be expected in the presence of NOM [39], depending on the type of organic matter present in the water.

Because it was found experimentally that $[OH]_{ssPBS} > [OH]_{ssSW}$, it suggests that OH radicals alone did not cause the enhanced inactivation of MS2 in SW, but that other reactive species contributed to this phenomena. Therefore, a CT calculation based on $[OH]_{ss}$ may be misleading in the presence of other photosensitizers. However, similar inactivation of MS2 was observed in PBS and SW under UV > 295 nm irradiation alone (data not shown) which rules out enhancements from UV photosensitizing effects (due to presence of NOM). These

results suggest a possible synergetic effect with other oxidizing species (variety of radical species ($^{\bullet}$OOR), for example, radical cations of aromatic structures, phenoxyl, and peroxyl radicals) formed by reaction between the $^{\bullet}$OH radicals (originated by the UV/H_2O_2 process) and NOM in the SW. This hypothesis will be investigated in future studies by adding OH radical scavenger (such as *t*-BuOH) to SW AOP system quantifying inactivation resulting from oxidizing species other than hydroxyl radicals.

3.6. *B. subtilis* spores

B. subtilis spores did not show any inactivation at any of the treatments used in this study (i.e H_2O_2, UV > 295 nm, and UV/H_2O_2). These results are in good agreement with previously published data. Riesenman and Nicholson [40] suggested that spores are resistant to H_2O_2 because the spore coat serves as a barrier for diffusion of the H_2O_2, or otherwise the coat proteins oxidize it before the H_2O_2 reaches the spore core. Spores exposed to UV radiation at longer wavelengths (UV > 290 nm, UVB, UVA) exhibit different DNA photochemistry and DNA repair responses as compared to 254 nm (UVC). For example, with spores exposed to UVB wavelengths, different photoproducts are formed compared to UVC with a shift in the major repair pathways [41]. Spore coat layers are thought to contribute to spore resistance during UVA and UVB (in sunlight) radiation but not to 254 nm UVC radiation [40]. Therefore, spores exposed to wavelengths above 295 nm are likely very UV resistant, as found in this study (data not shown). As described above, the primary OH radical mechanism of inactivating microorganisms is via attack on their cell membrane or walls. Hence, the spore coat layers are expected to protect the spores against attack by OH radicals, as indicated in this study. Yet, Cho et al. [42] reported that under certain experimental conditions, OH radicals, produced by ozone disinfection, played a significant role in the inactivation of *B. subtilis* endospores in pH-controlled ozone-demand-free distilled water.

4. Conclusions

Hydrogen peroxide did not show any microbial activity as a disinfectant. *B. subtilis* spores were not inactivated by any of the treatments applied (i.e. UV > 295 nm, H_2O_2, and UV/H_2O_2). The sensitivity of the viruses to filtered UV ($\lambda > 295$ nm) followed a decreasing order of T4 > T7 > MS2, where MS2 did not show any inactivation when exposed to wavelengths above 295 nm. The larger impact of UV/H_2O_2 was observed for MS2, while UV/H_2O_2 did not result in an additive effect on T4 inactivation, and only slight additional effect on T7 as compared to UV > 295 nm. The *E. coli* bacteria was very sensitive to filtered UV with a slight additional impact by UV/H_2O_2. Steady state hydroxyl radical CT for achieving 0.5 log inactivation of T7, *E. coli*, and MS2 were 1.47×10^{-13}, 1.47×10^{-13}, and 1.18×10^{-13} M min, respectively. UV/H_2O_2 efficiency depends on the microorganism and water type. These results indicate that for an UV based AOP process, the disinfection due to the presence of OH radicals is very small compared to the damage from the UV irradiation, although for viruses, there may be some oxidative enhancements that can assist disinfection efficacy.

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