ROLES OF SINGLET OXYGEN AND TRIPLET EXCITED STATE OF DISSOLVED ORGANIC MATTER FORMED BY DIFFERENT ORGANIC MATTERS IN BACTERIOPHAGE MS2 INACTIVATION

BY

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THESIS

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ABSTRACT

Inactivation of bacteriophage MS2 by reactive oxygen species (ROS) produced by irradiation with simulated sunlight, containing visible light and ultraviolet light with wavelengths greater than 320 nm, was investigated. The results from experiments with synthetic sensitizers Rose Bengal, 3'methoxyacetophenone, and nitrite suggested that singlet oxygen ($^1O_2$), triplet excited state of dissolved organic matter (^{3}DOM*), and hydroxyl radicals (·OH) could separately inactivate bacteriophage MS2. Natural sources of sensitizers included purified DOM isolates obtained from wastewater and river waters and three water samples collected from Singapore River, Stamford Canal, and Marina Bay Reservoir in Singapore. Linear correlations were found between MS2 inactivation rate constants (k_{obs}) and the photo-induced reaction rate constants of 2,4,6-trimethylphenol (TMP), a probe compound that previously had been shown to react mainly with ^{3}DOM*. Linear correlations between MS2 k_{obs} and $^1O_2$ concentrations were also found for both purified DOM isolates and natural water samples. These correlations, along with data from quenching experiments and experiments with synthetic sensitizers suggest that both $^1O_2$ and ^{3}DOM* play important roles in MS2 inactivation. Linear correlations between MS2 k_{obs} and Specific Ultraviolet Absorption determined at 254 nm ($SUVA_{254}$) were also found for both purified DOM isolates and natural samples. These results suggest the potential use of TMP as a chemical probe and $SUVA_{254}$ as an indicator for virus inactivation in natural and purified DOM water samples.

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CHAPTER 1
INTRODUCTION

In developing countries, only a fraction of the wastewater produced by the population is treated. For example, in Latin America, less than 15% of the wastewater collected in cities and towns with sewer systems is treated prior to discharge (Pan American Health Organization, 2001). Unfortunately, highly developed countries still have water quality problems due to leaking sewage, discharges of inadequately treated sewage into drinking water sources, urban runoff, and combined sewer flows. Aw and Gin (2011) detected at least one enteric virus in 83.3% of the samples collected from surface waters around Singapore. In many places where untreated wastewaters contaminate drinking water sources due to insufficient infrastructure or improper maintenance, chances of acquiring waterborne diseases are high (Friedler, 2004). However, since conventional water treatment technologies are not always capable of removing or inactivating viruses (Mi et al., 2005; Sirikanchana et al., 2008a; Sirikanchana et al., 2008b), the disinfection of viruses through solar radiation has been suggested as a solution to this water quality problem (Davies-Colley et al., 1999; Kohn and Nelson, 2007; Love et al., 2010).

As solar disinfection has become more common in recent years, the number of studies investigating inactivation mechanisms of microorganisms through solar disinfection has increased (Davies-Colley et al., 1999; Kohn et al., 2007; Romero et al., 2011). The indirect exogenous inactivation of bacteriophage MS2 involves the absorption of sunlight between UVB and visible light wavelengths by exogenous sensitizers, such as dissolved organic matter (DOM), which then catalyze the production of reactive oxygen species (ROS) responsible for damaging internal targets, such as DNA (Davies-Colley et al., 1999). Different types of ROS are produced upon DOM excitation, including singlet oxygen ($^1O_2$), hydroxyl radical ($\cdot OH$), and triplet excited

state of dissolved organic matter (^{3}DOM*) (Canonica and Freiburghaus, 2001; Davies-Colley et al., 1999; Kohn and Nelson, 2007). Numerous studies have shown the importance of exogenous sensitizers, such as DOM and trace metals, in bacteriophage inactivation due to their ability to produce different ROS upon excitation (Davies-Colley et al., 1999; Kohn et al., 2007; Kohn and Nelson, 2007; Romero et al., 2011). For example, Kohn and Nelson (2007) showed the importance of $^1O_2$ in MS2 inactivation, while Nieto-Juarez et al. (2010) suggested ·OH as an important ROS for MS2 inactivation through the addition of iron and copper to produce ·OH via photo-Fenton-like processes. However, the role of other photo-oxidants formed upon photosensitization of DOM, such as ^{3}DOM*, in MS2 inactivation is still unidentified due to the complex chemical composition of DOM (Canonica and Freiburghaus, 2001).

The goal of this study was to elucidate the role of ^{3}DOM* in the indirect exogenous inactivation of MS2 bacteriophage while using both purified DOM isolates and natural water samples. The indirect exogenous mechanism was chosen because the elevated concentrations of exogenous sensitizers, such as DOM, are expected in surface waters and wastewaters. MS2 was selected as the target virus since it has long been used as a surrogate for human enteric viruses, due to their similar size and morphology (Fisher et al., 2012; Havelaar et al., 1991; Havelaar et al., 1993; Kohn et al., 2007; Kohn and Nelson, 2007). In addition, a better understanding of MS2 inactivation and the ROS that enhance such inactivation may allow the identification of the DOM moieties that are responsible for the production of these ROS and the resulting virus inactivation.

CHAPTER 2
MATERIALS AND METHODS

2.1 Chemicals and Reagents

The following chemicals were of the purest grade available from commercial sources and were used as received: furfuryl alcohol (FFA, 98% Acros Organics); phenol (99+% Acros Organics); 2, 4, 6-trimethylphenol (TMP, 99% Sigma-Aldrich); sorbic acid (*trans, trans*-hexadienoic acid, t, t-HDA, 99% Acros Organics). Synthetic sensitizers: 3'-methoxyacetophenone (3'-MAP, 97% Acros Organics); Rose Bengal (95% dye content, Sigma-Aldrich); sodium nitrite (98.5% Acros Organics). Quenching agents: L-Histidine (98% Acros Organics); sodium formate (≥99% MP Biomedicals). Deuterium oxide (D_2O, 99.8% Acros Organics) was also used as received.

2.2 Model Viruses

MS2 bacteriophage (ATCC 15597-B1) was replicated and purified as described in previous studies (Gutierrez et al., 2009). Briefly, *Escherichia coli* (ATCC 15597) was grown in tryptic soy broth solution and inoculated with a stock of 10^{12} plaque forming units (PFU) per mL of MS2. The MS2 was purified by sequential centrifugation (Eppendorf centrifuge 5416) and microfiltration through 0.2 µm low-protein-binding polycarbonate track-etched membrane (Whatman Nucleopore, USA) to remove cell debris. The filtered MS2 stock was concentrated using a 100-kDa membrane (Koch Membranes, USA) in a Millipore ultrafiltration unit (Whatman Nucleopore, USA) and rinsed with a sterilized 1mM NaCl solution. The final stock was stored at 4°C at a final concentration of approximately 10^{12} PFU/mL and further diluted into several centrifuge tubes to concentrations of 10^9 PFU/mL to use in experiments.

2.3 Selection of Aquatic Dissolved Organic Matter and Effluent Organic Matter

The purified DOM isolates include three natural organic matter (NOM) isolates extracted from water samples collected from Suwannee River (USA), Loire River and Blavet River (France), two effluent organic matter (EfOM) isolates extracted from effluent wastewater samples collected from a Jeddah wastewater treatment plant (Saudi Arabia), and an isolate extracted from supernatant water from a membrane bioreactor (MBR) in Australia's North Head wastewater treatment plant. See Table 1 for sample designation. Both NOM and EfOM isolates were studied because NOM isolates from river water have a more pronounced terrestrial origin (i.e., allochthonous origin), while EfOM isolates originate from microbial activity (i.e., autochthonous origin) in domestic wastewater. Hydrophobic (HPO) NOM and EfOM fractions were isolated using XAD-8 resin following the protocol described by Croue et al. (1999) and Lee et al., (2006). It is important to note that HPO fractions represent only 40 to 50% of the DOM content of treated wastewater and river water, and also incorporate a major part of the UV absorbing moieties (i.e., aromatic structures) (Croue et al., 1999). Purified DOM solutions were made by adding the freeze-dried powder of the NOM or EfOM isolates to 1 mM bicarbonate buffer to maintain pH at 8. The solutions were then filtered through 0.2 μm low-protein-binding polycarbonate track-etched membranes (Whatman Nucleopore, USA).

Besides purified DOM isolates, natural water samples from Singapore, a place with a tropical climate, were also used. The natural water samples were collected in July, 2012 from three different sampling points located throughout Singapore: Singapore River (TOC = 3.30 mg/L, pH = 8.33, salinity = 372 ppm, alkalinity = 71.4 mg/L as $CaCO_3$), Stamford Canal (TOC = 3.09 mg/L, pH = 8.20, salinity = 120 ppm, alkalinity = 53 mg/L as $CaCO_3$) and Marina Bay Reservoir (TOC = 3.18 mg/L, pH = 8.72, salinity = 420 ppm, alkalinity = 70.4 mg/L as $CaCO_3$).

These sampling points were selected due to their different characteristics: the first is a major river in the southern part of Singapore, the second is a canal of a highly urbanized water catchment, and the third is a connecting freshwater reservoir. Water samples were collected with autoclaved and sample-rinsed amber bottles and transported on ice for immediate processing. Upon collection, the water samples were filtered through 0.2 μm low-protein-binding polycarbonate track-etched membranes (Whatman Nucleopore, USA) to remove particulate matter, and stored in the dark at 4°C until analysis. Salinity was measured using Eutech Instruments PC2700, and alkalinity was measured through titration. TOC and UV absorbance were measured using a Shimadzu TOC analyzer and a Shimadzu UV-1800 spectrophotometer, respectively.

2.4 Experimental Setup for Virus Inactivation

Solar disinfection experiments with purified DOM isolates were performed using an Atlas Suntest (r) XLS + photosimulator (Chicago, IL) equipped with a xenon arc lamp. The solar simulator included a filter (Atlas MTS, Cat. 56052372), which attenuated the irradiance below 320 nm and was used for all irradiation experiments. Experiments performed in Singapore with natural water samples were conducted using an Atlas Suntest CPS+ from Material Testing Technology GmbH (Singapore) equipped with a xenon arc lamp. This solar simulator was fitted with the same filter to block the transmission of wavelengths below 320 nm and was used for all irradiation experiments. In both setups, additional filters (Newport, FSQ-WG320) were used and placed on top of the reactors, to completely eliminate the UVB portion of the spectra responsible for direct inactivation, as measured in a previous study (Romero et al., 2011). The solar simulator intensities were set to 400 W m^{-2} and were maintained constant throughout all experiments. All reactors used were painted black to prevent light reflection and submerged in a

circulating water bath set at 40°C. This temperature was kept constant in all experiments for the following reasons. First, since the inactivation of MS2 is temperature dependent and has been shown to proceed faster at higher temperatures (Kohn and Nelson, 2007; Romero et al., 2011), 40°C was chosen to accelerate the process of inactivation. Also, it was chosen because the inactivation data for MS2 bacteriophage are compared with the inactivation data for rotavirus obtained at 40°C (Kohn and Nelson, 2007; Romero et al., 2011). Such high temperatures can be found in the upper layers (4-30 cm depths) of selected water bodies, as it has been shown that temperatures can fluctuate between 0 to 35°C, depending on the season, with variations of up to 14°C (Gu et al., 1996). In addition to having constant temperature, all sample solutions were stirred using magnetic stir bars set to 130 rpm, to maintain homogeneity. Dark controls were included for all inactivation experiments. They were prepared under the same conditions as for the irradiated samples but were covered using aluminum foil.

The reactors for inactivation experiments with purified DOM isolates contained 1 mM sodium bicarbonate ($NaHCO_3$) buffer and an initial concentration of 10^7 PFU/mL of MS2. DOM stock solutions (approximately 200 mg C/L) were added to the reactors at a final concentration of 20 mg C/L. Given that bacteriophage MS2 does not aggregate in 1mM $NaHCO_3$ buffer, but significantly does so in phosphate buffer (PBS) (Yuan et al., 2008), and that aggregation may influence virus infectivity, 1 mM $NaHCO_3$ buffer was used for all purified DOM experiments. For inactivation experiments with natural water samples, filtered water samples were added to the reactors and MS2 was added at the same concentration as for the purified DOM reactors. All reactors contained an initial sample volume of 8 mL. Sample aliquots (200 µL) were taken at regular time intervals, diluted in 1 mM $NaHCO_3$ buffer, and plated following the double agar

layer procedure (Adams, 1959). Several dilution factors were used, and the dilution that resulted in plate counts ranging from 10 to 300 plaques was selected.

Quenching experiments were also performed to determine the contribution of each ROS to exogenous MS2 inactivation. Quenchers are known to suppress the production of the ROS under consideration, thus the effect of ROS depletion on MS2 inactivation can be determined. In this study, L-Histidine was used as a quencher for $^1O_2$, and formate for ·OH, as done previously (Davies-Colley et al., 1999; Kohn and Nelson, 2007). For quenching experiments, a setup similar to the one for inactivation experiments was used. The reactors for L-Histidine experiments contained 1 mM $NaHCO_3$, an initial concentration of 10^7 PFU/mL of MS2, 20 mg C/L of DOM, and 20 mM of L-Histidine. The same solution composition was used for ·OH quenching experiments. However, 50 mM of sodium formate was added instead of 20 mM of L-Histidine. Sample aliquots (200 µL) were also taken at regular time intervals and enumerated as for the virus inactivation experiments. Due to time constraints, quenching experiments could not be performed with the Singaporean natural water samples.

Experiments with 3'methoxyacetophenone (3'-MAP), Rose Bengal (RB), and nitrite as synthetic sensitizers were also performed. Different synthetic sensitizers have been shown to produce higher concentrations of certain ROS, thus synthetic sensitizers can help determine the effects of one specific ROS at a time. In this study, 3'-MAP, RB, and nitrite were used as sensitizers for ^{3}DOM*, $^1O_2$, and ·OH, correspondingly, as done previously (Canonica et al., 1995; Davies-Colley et al., 1999; Gerecke et al., 2001; Kohn and Nelson, 2007; Mack and Bolton, 1999). Different reactors were prepared for each synthetic sensitizer. The reactors contained 1 mM $NaHCO_3$, 10^7 PFU/mL of MS2 and either 15 mg/L, 0.5 µM or 2-3.3 mg/L of 3'-

MAP, RB or nitrite, respectively. 200 µL samples were taken at specific time intervals, diluted, and plated as in the previous cases.

2.5 Experiments with Chemical Probes

The steady-state concentrations of $^1O_2$ and ·OH were indirectly measured by monitoring the decay of probe compounds furfuryl alcohol (FFA) and phenol, respectively (Haag and Hoigne, 1986; Kochany and Bolton, 1991). Applied probe compounds have known quenching rate constants ($1.2 \cdot 10^8 M^{-1}s^{-1}$ for FFA; $1.4 \cdot 10^{10} M^{-1}s^{-1}$ for phenol). Initial concentrations of probe compounds in the reactors were 10 µM for phenol and 50 µM for FFA. Reaction solutions were buffered to pH 8 with 1 mM $NaHCO_3$ buffer. Reactors were irradiated in the solar simulator under the same conditions as described for inactivation experiments. Sample aliquots (600 µL) were taken at regular intervals and transferred to 2 mL amber glass vials with a crimp seal. The decay of probe compounds was analyzed immediately after sampling by a reverse-phase HPLC 1200 Series (Agilent Technologies), equipped with an XDB-C18 column (Agilent Technologies). The method for phenol was: 60% Methanol/ 40% Water, flow rate = 1mL/min, stop time = 2.5 min; and that for FFA was: 40% acetonitrile/ 60% Nanopure water and 0.1% formic acid, flow rate = 0.41 mL/min, stop time = 5.3 min. UV detection wavelengths were set at 268 nm for phenol and 216 nm for FFA.

Steady-state concentrations of ^{3}DOM* produced by purified DOM isolates and synthetic sensitizers were measured by using sorbic acid (*trans, trans*-hexadienoic acid, t, t-HDA) as a probe compound (Grebel et al., 2011). Photo-products of sorbic acid after reaction with ^{3}DOM* were analyzed using the HPLC with a C18 column and a UV detector set at 254 nm. Samples were eluted with 85% 30 mM acetate buffer at pH 4.75 and 15% acetonitrile at a flow rate of 1.2 mL/min. After a stop time of 9.5 min, four isomer peaks were observed and analyzed as

described elsewhere (Grebel et al., 2011). We were unable to perform sorbic acid and phenol experiments with the natural water samples due to the presence of multiple unknown, unresolved HPLC peaks.

For the 2,4,6-Trimethylphenol (TMP) degradation experiments, similar conditions were used. Solutions were prepared with 1mM $NaHCO_3$ buffer, 20 mg C/L of DOM, and 10 µM of TMP. For experiments with natural samples, filtered solutions of the water samples were added to the reactors and 10 µM of TMP was subsequently added. Reactors were irradiated in the solar simulator under the same conditions as described for inactivation experiments. Samples (600 µL) were collected at regular time intervals in 2 mL amber glass vials and stored in the dark until analysis. The decay of TMP was quantified using the HPLC equipped with a C18 column with a mobile phase of 70% methanol and 30% water with acetic acid to control pH. The mobile phase was pumped at 0.9 mL/min. TMP was detected at 275 nm wavelength.

Dark controls were also included for all chemical probe experiments to confirm that other degradation pathways, such as hydrolysis and non-photolytic oxidation reactions, were not responsible for the probes' decays.

2.6 Kinetic Data Analyses

Given that MS2 inactivation follows a first order reaction with respect to time (Davies-Colley et al., 1999; Kohn and Nelson, 2007), first order rate constants, k_{obs} (h^{-1}), were determined by plotting ln (PFU/mL) versus time (h) and obtaining the slope. Linear regression was used to determine MS2 k_{obs} values and their corresponding 95% confidence intervals (CI) (Neter et al., 1990). No inactivation was observed in all dark controls, i.e., k_{obs} values for MS2 in the dark were not significantly different from zero ($p>0.05$). TMP decay also follows a first order reaction with respect to time (Canonica and Freiburghaus, 2001), thus TMP k_{obs} values were determined

as for MS2 k_{obs}. TMP k_{obs} and their corresponding 95% CI were also obtained using linear regression. Similar to inactivation rates in dark reactors, no significant probe degradations were observed in the dark, i.e., k_{obs} values for dark conditions were not significantly different from zero ($p>0.05$). To account for differences in light attenuation by the different sensitizers, inactivation rate constants for samples containing purified DOM isolates or natural water were adjusted using a light screening correction factor, as done previously (Kohn and Nelson, 2007; Romero et al., 2011). Briefly, the correction factor accounts for solution depth and UV absorption by the different sensitizers. The absorbance spectra from 280 nm to 400 nm of each solution subjected to irradiation experiments were taken. The correction factor is the ratio of the rate of light absorption at the optically thin surface layer to that over the entire solution depth. The former is calculated as the summation of the product of absorbance times light irradiance at a given wavelength. The latter is calculated as the summation of the product of absorbance times light irradiance at the bottom of the reactor. Statistical t-tests were performed to determine whether the MS2 k_{obs} in the synthetic sensitizer reactors were significantly different from the MS2 k_{obs} obtained from the control reactors ($p<0.05$) (Neter et al., 1990). In addition, multiple linear regression analysis was used to compare the slopes of the MS2 k_{obs} versus TMP, $SUVA_{254}$, and $^1O_2$ plots for the purified DOM isolates and natural water samples, and confirm the statistical differences between each other (Neter et al., 1990). It is important to point out that most conditions from the experiments mentioned in this section were tested in triplicate and similar trends were obtained in all cases. In the case of duplicate experiments, both values were reported.

CHAPTER 3
RESULTS AND DISCUSSION

3.1 MS2 Inactivation Rate Constants by Synthetic Sensitizers

To investigate the role of each ROS, i.e., ^{3}DOM*, $^1O_2$, and ·OH, in MS2 inactivation, experiments with synthetic photosensitizers were performed. The sensitizers and their concentrations were selected according to previous studies. Specifically, 3'methoxyacetophenone (3'-MAP) at 0.01 mM was chosen as a model sensitizer for ^{3}DOM*, as done previously (Canonica et al., 1995; Gerecke et al., 2001). Similarly, experiments were conducted with 0.5 μM of Rose Bengal (RB) and both 0.04 mM and 0.72 mM of nitrite (NO_2^-) as model sensitizers for $^1O_2$ and ·OH, respectively (Kohn and Nelson, 2007; Mack and Bolton, 1999). Concentrations of ^{3}DOM*, $^1O_2$, and ·OH produced by the synthetic sensitizers upon irradiation are shown in Table 1.

Table 1: **Radical and trace metal concentrations of the DOM sources**

DOM source	Radical Concentrations			$SUVA_{254}$ ($L \cdot mg^{-1}C \cdot cm^{-1}$)	Trace Metal Concentration ($\mu g \cdot mg^{-1}C$)	
	$[^1O_2]$ (pM)	$[^3DOM^*]$ (fM)	[·OH] (fM)		Fe	Cu
SWRHPO (hydrophobic fraction from Suwannee River NOM)	$(9.00 \pm 4.10) \cdot 10^{-2}$	$(1.60; 1.41) \cdot 10^{-1}$	$(2.75 \pm 0.60) \cdot 10^{-1}$ $(4.08 \pm 0.75) \cdot 10^{-1}$	4.60	1.31	0.01
EfOMHPO (hydrophobic fraction from effluent OM)	$(7.21 \pm 2.57) \cdot 10^{-2}$	$(4.71; 4.24) \cdot 10^{-1}$	$(6.26 \pm 2.40) \cdot 10^{-1}$ $(4.61 \pm 0.89) \cdot 10^{-1}$	2.90	ND	0.09
BRHPO (hydrophobic fraction from Blavet NOM)	$(17.71 \pm 2.65) \cdot 10^{-2}$	$(2.25) \cdot 10^{-1}$	$(3.62 \pm 0.72) \cdot 10^{-1}$	4.30	2.02	0.01
LRHPO (hydrophobic fraction from Loire NOM)	$(9.51 \pm 1.75) \cdot 10^{-2}$	$(3.79; 2.54) \cdot 10^{-1}$	$(4.35 \pm 1.00) \cdot 10^{-1}$	3.30	ND	0.36
EfOMTPI (transphilic fraction from effluent OM)	$(4.30 \pm 1.91) \cdot 10^{-2}$	$(4.17; 3.67) \cdot 10^{-1}$	$(3.20 \pm 1.83) \cdot 10^{-1}$	1.90	ND	0.11
MBRHPO (hydrophobic fraction from MBR supernatant)	$(4.97 \pm 1.05) \cdot 10^{-2}$	$(3.20; 2.40) \cdot 10^{-1}$	$(2.42 \pm 1.89) \cdot 10^{-1}$ $(4.71 \pm 0.48) \cdot 10^{-1}$	2.28	ND	0.07
3'-MAP (15 mg/L or 0.01mM)	NA	$(18.27; 16.90; 44.40) \cdot 10^{-1}$	NA	6.9	NA	NA
Rose Bengal (0.5 μM)	$(364.6 \pm 67.6) \cdot 10^{-2}$ $(385.3 \pm 36.3) \cdot 10^{-2}$	NA	$(31.73 \pm 18.01) \cdot 10^{-1}$ $(27.73 \pm 21.69) \cdot 10^{-1}$	17.9	NA	NA
Nitrite (2 mg/L or 0.04mM)	$(3.93 \pm 1.32) \cdot 10^{-2}$	NA	$(12.39 \pm 8.86) \cdot 10^{-1}$	NA	NA	NA
Nitrite (3.3 mg/L or 0.72 mM)	$(3.92 \pm 1.02) \cdot 10^{-2}$	NA	$(16.47 \pm 12.44) \cdot 10^{-1}$	NA	NA	NA
Singapore River	$(2.21 \pm 0.22) \cdot 10^{-2}$	NA	NA	2.12	NA	NA
Stamford Canal	$(1.27 \pm 0.12) \cdot 10^{-2}$	NA	NA	1.52	NA	NA
Marina Bay Reservoir	$(2.74 \pm 0.71) \cdot 10^{-2}$	NA	NA	2.20	NA	NA

ND: not detectable, NA: not available.

As shown in Table 2, MS2 k_{obs} values in the 3'-MAP reactor are statistically higher than those in the control ($p<0.05$), which demonstrates that MS2 inactivation is enhanced by the presence of ^{3}DOM* in solution. The results shown in Table 2 also reveal that MS2 k_{obs} values for RB (MS2 $k_{obs} = 4.55 \pm 0.86$ h^{-1}) and 2 mg/L of NO_2^- (MS2 $k_{obs} = 0.70 \pm 0.31$, 0.74 ± 0.32 h^{-1}) are also statistically higher than the control ($p<0.05$ in both cases). This implies that both $^1O_2$ and ·OH inactivate MS2, as does ^{3}DOM*. Similar results were reported by Kohn and Nelson (2007), where solutions of 0.5 µM of RB showed rapid MS2 inactivation (5.6 ± 1.2 h^{-1} in their study vs. 4.55 ± 0.05 h^{-1} in our study), which again shows that $^1O_2$ is involved in MS2 inactivation. In addition, Mamane et al. (2007) found that an additional two log inactivation of MS2 occurred with combined treatment by UVC and H_2O_2, which brings about oxidation by the generation of ·OH. Our study, however, used NO_2^- as a sensitizer for ·OH since it has long been known that the photolysis of NO_2^- and nitrate (NO_3^-) solutions result in ·OH formation (Mack and Bolton, 1999). It should be noted that although RB produced higher concentrations of ·OH than both NO_2^- solutions, RB's production of $^1O_2$ was much higher than its ·OH production, thus the effect of ·OH in RB solutions can be neglected. With this, we can conclude that the increased concentrations of ^{3}DOM*, $^1O_2$, and ·OH from these sensitizers enhanced MS2 inactivation, and that inactivation may be due to the synergistic effect of ^{3}DOM*, $^1O_2$, and ·OH in solution, rather than $^1O_2$ alone.

Table 2: MS2 inactivation rate constants and TMP degradation rate constants by synthetic sensitizers

Condition	MS2 k_{obs} (h^{-1})	TMP k_{obs} (h^{-1})
Rose Bengal (0.5 µM)	4.55 ± 0.86 (N=3)	1.62 ± 0.25 (N=3)
3'-MAP (0.01 mM)	0.72 ± 0.05 (N=4)	1.05 ± 0.19 (N=3)
NO_2^- (0.04 mM)	0.70 ± 0.31 0.74 ± 0.32 (N=2)	0.21 ± 0.03 (N=4)
NO_2^- (0.72 mM)	0.82 ± 0.54 0.92 ± 0.82 (N=2)	0.21 ± 0.03 (N=4)
Sensitizer-free control (1 mM $NaHCO_3$)	0.41 ± 0.10 (N=16)	0.13 ± 0.05 (N=3)

3.2 ROS Formation by Purified DOM Isolates

Since it was shown that ^{3}DOM*, $^1O_2$, and ·OH enhance MS2 inactivation, concentrations of ^{3}DOM*, $^1O_2$ and ·OH ([^{3}DOM*], [$^1O_2$] and [·OH], respectively) produced by the different DOM isolates were measured. Sorbic acid (*trans*, *trans*-hexadienoic acid, t, t-HDA) was used as a probe for the quantification of [^{3}DOM*], as done previously (Grebel et al., 2011). The data shown in Table 1 demonstrates that all purified DOM isolates produced ^{3}DOM* upon irradiation, where the [^{3}DOM*] ranged between approximately $1.5 \cdot 10^{-16}$ - $4.5 \cdot 10^{-16}$ M. Studies performed by Grebel et al. (2011) showed that sorbic acid was a better suited probe for quantifying ^{3}DOM* than TMP due to the HDA isomerization that results from the interaction of t,t-HDA with organic triplets. Other oxidants, such as $^1O_2$ and ·OH, will not produce isomer products; therefore, quantification of these isomers guarantees that the only reaction pathway is that with ^{3}DOM*. According to Grebel et al. (2011), 15 mg C/L of Suwannee River NOM produced approximately 10^{-16} M [^{3}DOM*], which is comparable to the [^{3}DOM*] found in this study.

To determine [$^1O_2$] produced by the different purified DOM isolates and natural water samples, furfuryl alcohol (FFA) was used as a probe for $^1O_2$ quantification, as done previously (Haag and Hoigne, 1986; Kochany and Bolton, 1991; Kohn and Nelson, 2007; Mack and Bolton,

1999). The results showed that [$^1O_2$] were typically on the order of 10^{-13} and 10^{-14} M for both purified DOM isolates and natural samples. Similarly, Kohn and Nelson (2007) found [$^1O_2$] to be approximately 10^{-13} M in water samples from a waste stabilization pond (WSP) and 5×10^{-14} M in Fluka and Suwannee River humic acid samples. Finally, [·OH] produced by the different purified DOM isolates upon irradiation were quantified using phenol as a probe. The data in Table 1 shows that all samples produced [·OH] on the order of 10^{-16} M.

As shown in Table 3, when formate was used to quench [·OH] in SWRHPO, EfOMHPO, and LRHPO samples, we observed from 22 to 48% reduction in MS2 k_{obs}. In contrast, a study by Kohn and Nelson (2007) found no significant reduction in the MS2 inactivation rate constant when formate was added to the Fluka and Suwannee River humic acid samples, and to WSP samples. However, Kohn and Nelson (2007) did not find a measurable concentration of [·OH] in their DOM solutions and WSP samples, while we found [·OH] on the order of 10^{-16} M in this study (Table 1). Although research has found that ·OH enhances MS2 inactivation (Cho et al., 2011; Mamane et al., 2007; Nieto-Juarez et al., 2010), it has also been determined that the disinfection rate of ·OH may be slow due to the relatively slow diffusion of ·OH into viruses (Watts et al., 1995). For these reasons, this work did not aim to study the role of ·OH in MS2 inactivation, although we do acknowledge its contribution to inactivation. Instead, we focused on the roles of $^1O_2$ and ^{3}DOM* in the inactivation of MS2.

Table 3: Effects of quenching on MS2 inactivation

	Ratios of $k_{obs\text{-}D2O}/k_{obs\text{-}H2O}$ (ratios of singlet concentration in D_2O and in H_2O)	Ratios of $k_{obs\text{-}L\text{-}histidine}/k_{obs}$	Ratios of $k_{obs\text{-}formate}/k_{obs}$
SWRHPO	1.26 (6.46); 2.5 (4.38)	0.76	0.57; 0.73
EfOMHPO	4.8 (8.63)	0.47; 0.55	0.68; 0.78
BRHPO	2.95; 2.4 (4.25; 4.73)	0.69; 0.64	1.1; 1.04
LRHPO	2.75 (5.38)	0.27; 0.30	0.54; 0.52

3.3 Inactivation of MS2 Bacteriophage by Singlet Oxygen

Linear correlations ($R^2 = 0.86$ and $R^2 = 0.95$) were found between MS2 k_{obs} and $[^1O_2]$ in different purified DOM isolates containing 20 mg C/L and natural water samples (Figure 1). While the measured $[^1O_2]$ was typically on the order of 10^{-13} and 10^{-14} M for both purified DOM isolates and natural samples, MS2 k_{obs} in the natural water samples were approximately twice as high as those in purified DOM isolates. Consistent with findings by others (Davies-Colley et al., 1999; Kohn and Nelson, 2007), organic matter present in natural waters also enhances the exogenous inactivation, thus increases MS2 inactivation rates. Kohn and Nelson (2007) reported $^1O_2$ as the most important ROS involved in the inactivation of MS2 and concluded that it could be used as an indicator to estimate MS2 inactivation in water samples from a WSP. Similar to our study, high correlations ($R^2 = 0.90$) between MS2 k_{obs} and $[^1O_2]$ were found. However, a study by Kohn et al. (2007) showed that the observed inactivation rates, k_{obs}, did not increase linearly with the $^1O_2$ concentrations in the aqueous bulk, $[^1O_2]_{bulk}$, but appeared to saturate at higher NOM concentrations, which in turn is associated with higher $[^1O_2]$. This phenomenon was particularly prevalent for Suwannee River and Fluka humic acid samples, where their k_{obs} values plateaued as NOM concentrations increased. Interestingly, the Kohn et al. (2007) study showed

that k_{obs} was almost fully governed by the [$^1O_2$] in close proximity to NOM as measured by chemiluminescent probes (Latch and McNeill, 2006). Similarly, the results reported by Hotze et al. (2009) and Badireddy et al. (2007) with photo-sensitized hydrophilic fullerenes also showed that due to extremely short lifetimes (between 3-4 μs), $^1O_2$ needed to be generated close to MS2 to enhance inactivation.

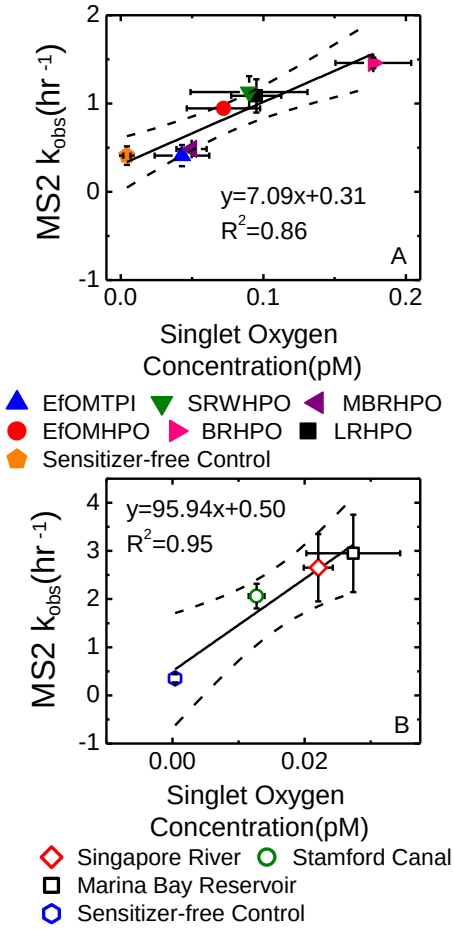

Figure 1. Dependence of MS2 k_{obs} on [$^1O_2$] in different purified DOM isolates (20 mg C/L) prepared in 1 mM sodium bicarbonate buffer (A) and natural water samples (~3 mg C/L) (B) at 40°C. All samples were irradiated with UVA and visible light. The plots are corrected for light screening and the error bars correspond to 95% confidence intervals. The confidence bands of 95% for the linear correlation are also presented.

In the presence of L-Histidine, a known $^1O_2$ quencher, the MS2 k_{obs} in the purified DOM isolates was 0.27-0.76 times the MS2 k_{obs} in the purified DOM isolates without L-Histidine

(Table 3). Similar results were reported by Kohn and Nelson (2007) where the MS2 k_{obs} in WSP water in the presence of L-Histidine was 0.57 times the MS2 k_{obs} in the WSP sample without L-Histidine. This decrease in inactivation due to $^1O_2$ quenching suggests that $^1O_2$ is important for MS2 inactivation. Additional experiments with D_2O were conducted since D_2O does not quench $^1O_2$, unlike H_2O. Consequently, reactors prepared with D_2O are expected to have higher concentrations of $^1O_2$ compared to H_2O, and thus the corresponding MS2 k_{obs} should also increase accordingly (Kohn and Nelson, 2007; Romero et al., 2011). For the D_2O experiments, both MS2 k_{obs} and $[^1O_2]$ were determined and compared to those obtained in H_2O reactors. The results presented in Table 3 shows that $[^1O_2]$ was approximately 4-8 times higher in D_2O reactors than in H_2O reactors. However, MS2 k_{obs} values were only 1.26-4.8 times higher in the D_2O reactors, suggesting the effect of other oxidants in MS2 inactivation. Similar results were reported by Kohn et al. (2007) where $[^1O_2]$ in different NOM solutions were enhanced 12-fold with 99.9% D_2O, while MS2 k_{obs} only increased between 3.0 and 10.3-fold. Similarly, a previous study by Romero et al. (2011) with Suwannee River NOM (SRNOM) showed that $[^1O_2]$ was 5 times higher in D_2O reactors than in H_2O reactors. Nevertheless, MS2 k_{obs} values for D_2O reactors were 2.4 times higher than those in the H_2O reactors. Results obtained by this and previous studies suggest that $^1O_2$ may only account for a fraction of the total inactivation of MS2. It is worth mentioning that the reaction of $^1O_2$ with bacteriophage MS2 is not likely to form compounds responsible for $^1O_2$ scavenging, hence the decreased MS2 k_{obs} values in D_2O. Given singlet oxygen's partial contribution to the inactivation of MS2, the following section will discuss the role of ^{3}DOM* on MS2 inactivation.

3.4 2,4,6-Trimethylphenol as a Probe Compound for MS2 Inactivation by ^{3}DOM*

After DOM, a ubiquitous photo-sensitizer in water, absorbs light, it is promoted to its first excited singlet state, ^{1}DOM*. ^{1}DOM* quickly decays to excited triplet state, ^{3}DOM*, due to ^{1}DOM*'s short lifetime. The ^{3}DOM* then deactivates by transferring its energy to dioxygen (O_2) molecules naturally present in water, which results in $^1O_2$ production (Sharpless, 2012; Zepp et al., 1985). To probe the presence of ^{3}DOM* in natural waters, the loss of TMP, a well-studied compound (Canonica and Freiburghaus, 2001; Canonica et al., 1995; Canonica and Laubscher, 2008), has commonly been used.

As shown in Table 2, TMP k_{obs} values for all three synthetic sensitizers were higher than the sensitizer-free control experiments ($p<0.05$ in all cases). This observation is similar to those for MS2 k_{obs} with synthetic sensitizers. In addition, the TMP k_{obs} values obtained for both purified DOM isolates and natural water samples typically ranged between 0.3 and 0.5 h^{-1}. Given that ^{3}DOM* may act as precursors of $^1O_2$, as mentioned above, TMP k_{obs} was correlated with the [$^1O_2$] produced in both purified DOM and natural water samples (Figure 2). The results showed high correlations ($R^2 = 0.91$ and $R^2 = 0.98$) in both cases, suggesting that the presence of $^1O_2$ is important for TMP oxidation. However, studies have shown that $^1O_2$ alone cannot be accountable for TMP oxidation by ROS in natural surface water (Aguer et al., 2005; Canonica and Freiburghaus, 2001; Canonica et al., 1995; Tratnyek and Hoigne, 1994). Specifically, a study by Tratnyek and Hoigné (1994) with natural water samples showed that TMP photo-oxidation occurs more rapidly than can be accounted for by $^1O_2$, which implies that TMP reacts first with other photo-oxidants besides $^1O_2$. In addition, Canonica et al. (1995) determined that photo transformations of phenols at conditions typical of natural surface waters occurred mainly as a non-singlet oxygen process. The same study also concluded that ·OH does not play an important

role in TMP degradation, but instead the reaction is mainly due to the ^{3}DOM* produced upon DOM excitation. Another study showed that at higher TMP concentrations, short lived ^{3}DOM* are the main contributors of TMP decay, whereas both short and longer lived ^{3}DOM* are responsible for TMP degradation at lower probe concentrations (Canonica and Freiburghaus, 2001). A more recent study conducted by Halladja et al. (2007) used furfuryl alcohol, a $^1O_2$ scavenger, and performed experiments with different concentrations of dissolved oxygen to show that ^{3}DOM* can oxidize TMP and also react with O_2 to form $^1O_2$. Therefore, the correlation shown in Figure 2 does not prove that $^1O_2$ is the ROS responsible for oxidizing TMP, but rather confirms that $^1O_2$ is involved in the process.

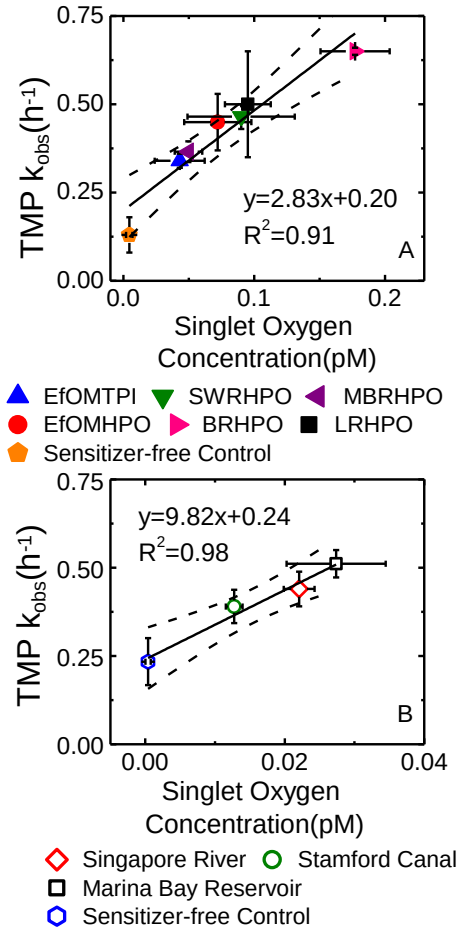

Figure 2. Involvement of $^1O_2$ in TMP degradation in different purified DOM isolates (20 mg C/L) prepared in 1 mM sodium bicarbonate buffer (A) and natural water samples (B). The plots are corrected for light screening and the error bars correspond to 95% confidence intervals. The confidence bands of 95% for the linear correlation are also presented.

As discussed above, MS2 was inactivated by the ^{3}DOM* produced by synthetic sensitizer, 3'-MAP, and $^1O_2$ alone did not account for MS2 inactivation. For this reason, TMP was used as a probe compound to investigate the role of ^{3}DOM* in MS2 inactivation. Linear correlations (R^2 = 0.74 and R^2 = 0.98) between MS2 k_{obs} and TMP k_{obs} in different purified DOM isolates and natural water samples were found (Figure 3). The linear correlations between MS2 k_{obs} and TMP k_{obs} suggest once more that $^1O_2$ might not be the only ROS involved in MS2 inactivation, but rather a synergistic effect of $^1O_2$ and ^{3}DOM*, an intermediate oxidant in $^1O_2$

formation, may be responsible for such inactivation. Additionally, the involvement of $^1O_2$ in both TMP degradation and MS2 inactivation could explain the correlation found between MS2 k_{obs} and TMP k_{obs}, shown in Figure 3.

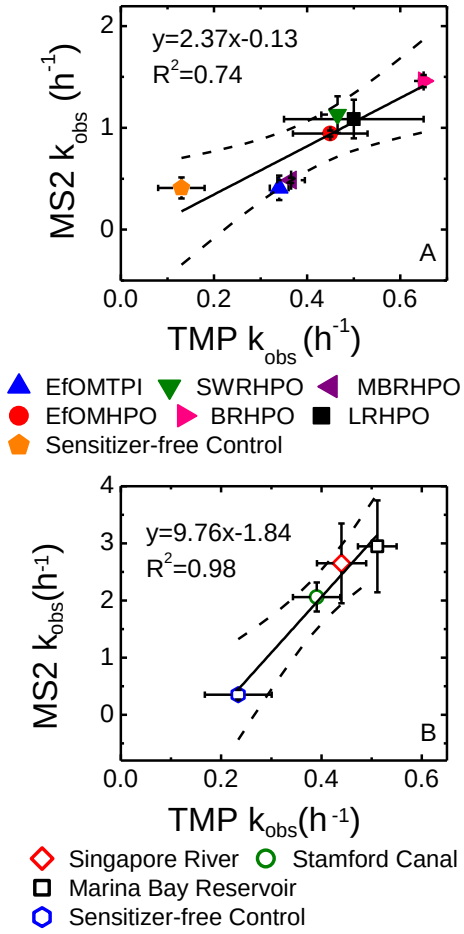

Figure 3. MS2 k_{obs} as a function of TMP degradation ([TMP_0] = 10 µM) in different purified DOM isolates (20 mg C/L) prepared in 1 mM sodium bicarbonate buffer (A) and natural water samples (B) at 40°C. All samples were irradiated with UVA and visible light. The plots are corrected for light screening and the error bars correspond to 95% confidence intervals. The confidence bands of 95% for the linear correlation are also presented.

In this study, 1 mM $NaHCO_3$ buffer was used for all experiments instead of PBS in order to prevent MS2 aggregation. However, it is well known that excited triplet state of a sensitizer (^{3}Sens*) reacts with bicarbonate anions present in solution to generate carbonate radicals ($CO_3^{\cdot-}$) (Canonica et al., 2005), which in turn are involved in competitive interactions with ·OH (Huang

and Mabury, 2000). Therefore, TMP degradation control experiments using 20 mg C/L EfOMHPO in both 20 mM PBS at pH 7.3 and 1 mM $NaHCO_3$ at pH 7.4 were conducted to prove that the presence of bicarbonate anions did not significantly influence the kinetics of TMP. Specifically, TMP k_{obs} values for solutions containing 1 mM $NaHCO_3$ and 20 mM PBS were $0.31 \pm 0.04\ h^{-1}$ and $0.28 \pm 0.04\ h^{-1}$, respectively. Student t-tests for these triplicate experiments showed that the TMP k_{obs} values were statistically similar ($p>0.05$).

3.5 Properties of DOM and MS2 inactivation

$SUVA_{254}$ data was determined for all purified DOM and natural water samples and correlated with their corresponding MS2 k_{obs} (Figure 4A). Linear correlations ($R^2 = 0.75$ and $R^2 = 0.99$) were found for both purified DOM isolates and natural water samples. However, since $SUVA_{254}$ values for the natural water samples were lower than for the purified DOM isolates, but the MS2 k_{obs} were significantly higher, the slopes of the linear regressions were significantly different from each other ($p<0.05$). This implies that MS2 inactivation is very sensitive to small variations of $SUVA_{254}$ in natural water samples. The $SUVA_{254}$ values obtained for all water samples were also correlated to their corresponding TMP k_{obs}, determined previously. As shown in Figure 4B, a linear correlation between the $SUVA_{254}$ values and TMP k_{obs} of both purified DOM and natural water samples ($R^2 = 0.84$) was found. This indicates that high DOC reactivity and high aromatic carbon content in NOMs result in increasing reactivity with TMP. Similar results were reported by Westerhoff et al. (1999), where high correlation coefficients were obtained between $SUVA_{254}$ and ozone decomposition, suggesting that increasing $SUVA_{254}$ values results in increasing reactivity with ozone.

While 254 nm is not present in solar UV spectrum, i.e., light used for this study, SUVA is used to characterize the organic matrix (aromatic vs. nonaromatic) because photo-sensitizers are aromatic in nature.

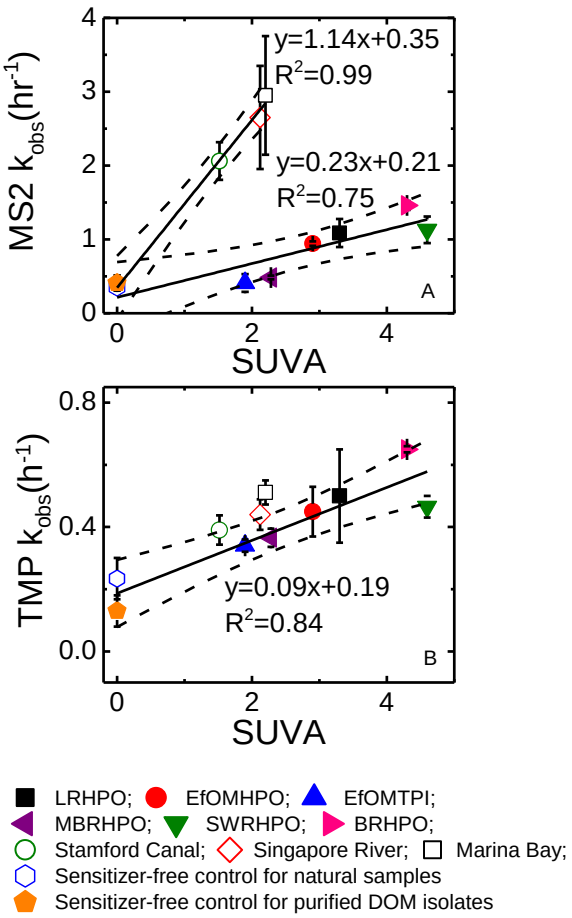

Figure 4. MS2 k_{obs} as a function of $SUVA_{254}$ in different purified DOM isolates (20 mg C/L) prepared in 1 mM sodium bicarbonate buffer and natural water samples (A). TMP k_{obs} as a function of $SUVA_{254}$ in different purified DOM isolates (20 mg C/L) prepared in 1 mM sodium bicarbonate buffer and natural water samples (B). The plots are corrected for light screening and the error bars correspond to 95% confidence intervals. The confidence bands of 95% for the linear correlation are also presented.

$SUVA_{254}$ has long been used as a surrogate measurement for dissolved organic carbon (DOC) aromaticity, which in turn is used as an indicator of DOC reactivity in a number of environmental processes (Traina et al., 1990; Westerhoff et al., 1999). Moreover, $SUVA_{254}$ has

also been found to be a good indicator of aromatic carbon content, a general characteristic of the molecules that compose DOC, for both soils and aquatic humic substances (Westerhoff et al., 1999). Therefore, higher $SUVA_{254}$ values suggests that increases in DOC reactivity and aromatic carbon content may lead to higher MS2 inactivation, as seen from Figure 4A. This indicates that higher $SUVA_{254}$ of DOM results in higher reactivity with MS2. In contrast to the purified DOM samples, whose $SUVA_{254}$ ranged from 1.9-4.6 L mg^{-1} m^{-1}, the natural water samples had low $SUVA_{254}$ values and ranged from 1.52-2.20 L mg^{-1} m^{-1}. Weishaar et al. (2003) also obtained $SUVA_{254}$ values for well-characterized organic matter isolates derived from different type of aquatic environments. These values ranged from 0.6-5.3 L mg^{-1} m^{-1}, which is in accordance with our study. Interestingly, the same study determined that $SUVA_{254}$ is affected by the presence of nitrate and iron in solution, and by pH as well. They also concluded that minor differences in UV absorption are observed for samples between pH 2.0 and 8.6. In our study, the pH for the purified DOM samples was controlled with sodium bicarbonate buffer at pH 8. However, the pH for the natural samples varied. Specifically, the values were 8.33, 8.20 and 8.72 for the Singapore River, Stamford Canal and Marina Bay Reservoir samples, respectively. This suggests that pH could have affected the $SUVA_{254}$ values obtained for the natural water samples. Additionally, Weishaar et al. (2003) determined that concentrations of iron (III) in the range commonly found in water samples, 0-0.5 mg L^{-1}, add from 0-0.04 cm^{-1} to the UV absorbance at 254 nm of water samples. Although iron concentrations were not measured for the natural water samples in our study, there is a possibility that $SUVA_{254}$ values were affected by iron concentrations present in solution. Nitrate (NO_3^-) was also shown to affect $SUVA_{254}$ values, but at concentrations approximately eight times those expected in surface waters (Weishaar et al.

2003). Therefore, it can be assumed that NO_3^- levels did not affect the $SUVA_{254}$ values in our study.

It is important to note, however, that although lower $SUVA_{254}$ were obtained for the natural water samples compared to purified DOM isolates, higher reactivity was observed for natural water. This can be seen by the significantly higher slopes of the natural water samples in Figures 1, 3, and 4A ($p<0.05$), where small increases in $[^1O_2]$, TMP k_{obs} and $SUVA_{254}$, respectively, resulted in high increases of MS2 inactivation rates. The difference in reactivity between purified DOM isolates and natural water samples may be due to differences in their composition. Another reason for this difference could be that the steady state production and consumption of ROS by DOM aromaticity favors natural waters as compared to the purified DOM isolates.

The $SUVA_{254}$ values obtained for all water samples were also correlated to the $^1O_2$ produced by the DOMs. The results in Figure 5A show a positive trend between the $SUVA_{254}$ values and $[^1O_2]$ produced by both purified DOM isolates and natural water samples. While the linear correlation may not be the right model to present the trend with $R^2 = 0.59$, and $p=0.076$, this positive trend indicates that high DOC reactivity, aromaticity, and aromatic carbon content in NOMs result in increased production of $^1O_2$, which in turn enhance MS2 inactivation. However, an opposite trend was observed for $[^3DOM^*]$ produced by the purified DOM isolates and their $SUVA_{254}$. As shown in Figure 5B, purified DOM isolates with low $SUVA_{254}$ produced higher $[^3DOM^*]$. Thus, $^3DOM^*$ is probably quenched, or alternatively consumed, to form $^1O_2$, as DOM reactivity, aromaticity and aromatic carbon content increases. Highest $[^3DOM^*]$ in the hydrophilic isolate EfOMTPI with lowest $SUVA_{254}$ may suggest the presence of highly reactive low aromatics. Previous research has shown that although aromatic structures of DOM can act

as photo-sensitizers and may enhance transformation rates of contaminants, it can also scavenge photo-oxidants and inhibit the excited triplet-induced transformation of certain aromatic compounds (Canonica and Laubscher, 2008; Wenk and Canonica, 2012). Specifically, Wenk and Canonica (2012) reported that DOMs from terrestrial sources with high aromaticity were better inhibitors than those from aquatic sources with low aromaticity. Since our results suggest that high DOM aromaticity leads to ^{3}DOM* quenching or consumption, $^{1}O_2$ could be the ROS responsible for MS2 inactivation in samples with higher $SUVA_{254}$, thus higher aromatic carbon content, whereas both $^{1}O_2$ and ^{3}DOM* could be the most important ROS for MS2 inactivation in samples with lower $SUVA_{254}$ and, therefore, lower aromatic carbon content.

Although $SUVA_{254}$ may be used as an indicator for DOC reactivity and aromatic carbon content of DOMs, it does not provide specific information about the compositional difference between the samples (Weishaar et al., 2003). This may be due in part to the UV spectroscopy's inability to determine the functional groups present in these materials (Weishaar et al., 2003). Therefore, future work will need to include the identification and characterization of the individual molecules within the DOMs that are responsible for producing ROS, specifically $^{1}O_2$ and ^{3}NOM*, since we have shown them to be the main ROS involved in MS2 inactivation.

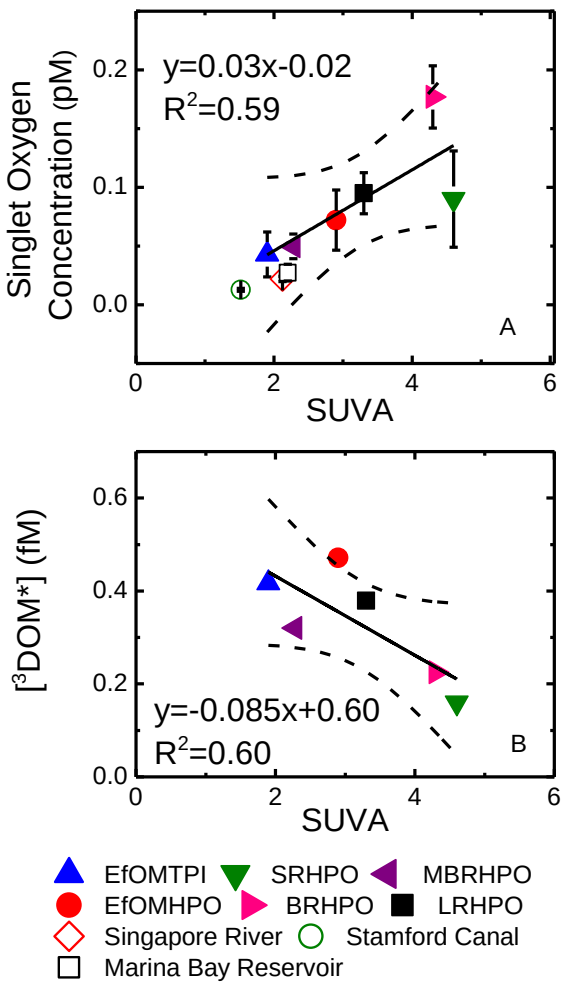

Figure 5. Singlet oxygen concentration as a function of $SUVA_{254}$ in different purified DOM isolates (20 mg C/L) prepared in 1 mM sodium bicarbonate buffer and natural water samples. Error bars correspond to 95% confidence intervals. The p value for the linear correlation is 0.076 (A). Correlation between [^{3}DOM*] and SUVA for purified DOM isolates (20 mg C/L). The p value for the linear correlation is 0.069 (B). The confidence bands of 95% for the linear correlation are also presented.

CHAPTER 4
CONCLUSIONS

The following conclusions resulting from this study may contribute to the understanding of both the MS2 inactivation mechanism and the DOM properties that enhance MS2 inactivation:

- The data reported here confirmed that $^1O_2$ may not be the only ROS involved in MS2 inactivation, but instead demonstrated that inactivation is mainly due to both $^1O_2$ and ^{3}DOM* present in the solution.
- The results from this study demonstrated that TMP k_{obs} may be used as an indicator for estimating MS2 inactivation rates, due to the linear correlations found between MS2 k_{obs} and TMP k_{obs} for both purified DOM isolates and natural water samples.
- $SUVA_{254}$ may also serve as an indicator to estimate MS2 inactivation rates. This was demonstrated by the linear correlations found between MS2 k_{obs} and $SUVA_{254}$ for both purified DOM isolates and natural water samples. DOM samples with higher $SUVA_{254}$ will result in higher reactivity with MS2, largely due to the high aromatic carbon content in the DOM.
- $^1O_2$ may be the most responsible ROS for MS2 inactivation in samples with high aromatic carbon content, whereas both $^1O_2$ and ^{3}DOM* may be important for MS2 inactivation in samples with low aromatic carbon content.
- The results presented here offer insight into the reactivity of DOMs that originate from different water sources.

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APPENDIX
EXPERIMENTAL RAW DATA

Table A.1. Raw data for purified DOM isolate samples

		MS2 k_{obs} (hr^{-1})	95% CI	$[^1O_2]$ (10^{-2}) pM	95% CI	$[^3DOM^*]$ (10^{-1}) fM	$[\cdot OH]$ (10^{-1}) fM	95% CI	TMP k_{obs} (hr^{-1})	95% CI	$SUVA_{254}$ L/(mg·m)
Purified DOM Samples	SR95HA	1.077	0.235	7.647	1.796	1.601	2.751	0.568	0.440	0.113	4.60
		1.297	0.282	5.557	1.089	1.412	4.079	0.747	0.506	0.133	
		1.239	0.166	7.828	2.701				0.434	0.033	
		1.198	0.285	14.956	2.383				0.480	0.146	
		1.148	0.737								
		0.800	0.560								
	Average	1.126		8.997		1.506	3.415		0.465		
	StDev	0.177		4.104		NA	NA		0.034		
	XAD8	0.951	0.033	6.776	0.642	4.706	6.257	2.405	0.490	0.069	2.90
		0.969	0.282	9.968	1.719	4.244	4.610	0.892	0.489	0.100	
		0.912	0.287	4.880	1.142				0.370	0.053	
	Average	0.944		7.208		4.475	5.434		0.450		
	StDev	0.029		2.571		NA	NA		0.084		
	Blavet	1.490	0.520	12.686	4.708	2.250	3.622	0.725	0.659	0.085	4.30
		1.490	0.640	22.737	10.440				0.639	0.106	
		1.393	1.364						0.660	0.234	
									0.638	0.106	
	Average	1.458		17.711		2.250	3.622		0.649		
	StDev	0.056		7.107		NA	NA		0.012		
	Loire	1.286	0.068	11.352	3.160	3.792	4.355	0.996	0.460	0.277	3.30
		0.909	0.211	7.870	1.088	2.540			0.371	0.079	
		1.065	0.151	9.299	2.057				0.667	0.091	
	Average	1.086		9.507		3.166	4.355		0.499		
	StDev	0.189		1.751		NA	NA		0.152		
	XAD4	0.543	0.125	4.297	1.911	4.169	3.204	1.826	0.368	0.052	1.90
		0.316	0.051			3.668			0.337	0.062	
		0.373	0.368						0.322	0.109	
	Average	0.411		4.297		3.919	3.204		0.342		
	StDev	0.118		NA		NA	NA		0.023		
	MBR	0.515	0.248	4.970	1.053	3.206	2.417	1.895	0.368	0.108	2.28
		0.476	0.051			2.403	4.715	0.483	0.399	0.039	
		0.464	0.194						0.329	0.072	
	Average	0.485		4.970		2.804	3.566		0.365		
	StDev	0.026		NA		NA	NA		0.035		

Table A.2. Raw data for synthetic sensitizers

		MS2 k_{obs} (hr^{-1})	95% CI	[$^1O_2$] (10^{-2}) pM	95% CI	[^{3}DOM*] (10^{-1}) fM	[·OH] (10^{-1}) fM	95% CI	TMP k_{obs} (hr^{-1})	95% CI
Synthetic Sensitizers	3'-MAP (15 mg/L or 0.01 mM)	0.690	0.560			18.271			1.005	0.074
		0.790	0.210			16.900			1.258	0.163
		0.706	0.314			44.400			0.887	0.377
		0.701	0.358							
	Average	0.722				26.524			1.050	
	StDev	0.046							0.189	
	Rose Bengal (0.5 µM)	4.920	2.280	364.590	67.563		31.732	18.006	1.348	0.151
		3.560	1.210	385.233	36.309		27.729	21.688	1.837	0.350
		5.160	2.120						1.727	0.149
	Average	4.547		374.911			29.731		1.621	
	StDev	0.863		NA			2.830		0.256	
	Nitrite (2 mg/L or 0.04 mM)	0.706	0.314	3.934	1.318		12.393	8.858	0.184	0.125
		0.737	0.323						0.214	0.059
									0.257	0.146
									0.194	0.124
	Average	0.721		3.934			12.393		0.212	
	StDev	NA		NA			NA		0.032	
	Nitrite (3.3 mg/L or 0.72 mM)	0.822	0.545	3.923	1.023		16.466	12.442	0.189	0.038
		0.918	0.825						0.214	0.059
									0.254	0.055
									0.194	0.124
	Average	0.870		3.923			16.466		0.213	
	StDev	NA		NA			NA		0.030	

Table A.3. Raw data for natural water samples

		MS2 k_{obs} (hr^{-1})	95% CI	$[^1O_2]$ (10^{-2}) pM	95% CI	TMP k_{obs} (hr^{-1})	95% CI	$SUVA_{254}$ L/ (mg · m)
Natural Water Samples	Singapore River	3.423	1.798	2.136	0.775	0.447	0.109	2.12
		2.063	0.850	2.523	0.244	0.507	0.199	
		2.468	1.124	2.046	0.315	0.402	0.060	
				2.118	0.712	0.404	0.112	
	Average	2.651		2.206		0.440		
	StDev	0.698		0.215		0.049		
	Stamford Canal	2.252	1.184	1.258	0.658	0.366	0.248	1.52
		1.772	1.014	1.157	0.496	0.455	0.164	
		2.160	1.101	1.405	0.460	0.392	0.016	
						0.347	0.014	
	Average	2.061		1.273		0.390		
	StDev	0.255		0.125		0.047		
	Marina Bay Reservoir	3.858	1.004	3.721	0.982	0.527	0.238	2.20
		2.331	1.231	2.767	0.290	0.526	0.244	
		2.659	0.822	2.107	0.116	0.454	0.037	
				2.353	0.416	0.538	0.203	
	Average	2.949		2.737		0.511		
	StDev	0.804		0.710		0.039		

Table A.4. Raw data for quenching experiments

	MS2 $k_{obs\text{-}Lhistidine}$ (hr^{-1})	MS2 $k_{obs\text{-}Formate}$ (hr^{-1})	MS2 k_{obs} (hr^{-1})	MS2 $k_{obs\text{-}Lhistidine}$/MS2 k_{obs}	MS2 $k_{obs\text{-}Formate}$/MS2 k_{obs}
SR95 HA	0.40	0.30	0.52	0.76	0.57
		0.38			0.73
XAD8	0.18	0.26	0.38	0.47	0.68
	0.21	0.30		0.55	0.78
Blavet	0.45	0.73	0.66	0.69	1.10
	0.52	0.68	0.79	0.65	1.04
Loire	0.11	0.22	0.41	0.27	0.54
	0.12	0.21		0.30	0.52

Table A.5. Raw data for D2O experiments

	MS2 $k_{obs\text{-}D2O}$ (hr^{-1})	MS2 $k_{obs\text{-}H2O}$ (hr^{-1})	MS2 $k_{obs\text{-}D2O}$/MS2$k_{obs\text{-}H2O}$	$[^1O_2]_{D2O}$ (M)	$[^1O_2]_{H2O}$ (M)	$[^1O_2]_{D2O}$ / $[^1O_2]_{H2O}$
SR95 HA	1.45	1.15	1.26	5.06E-13	7.83E-14	6.46
	2.00	0.80	2.50	6.56E-13	1.50E-13	4.38
XAD8	1.78	0.37	4.80	4.21E-13	4.88E-14	8.63
Blavet	1.49	0.51	2.94	1.55E-12	3.65E-13	4.25
	1.58	0.66	2.40	1.95E-12	4.12E-13	4.73
Loire	1.11	0.40	2.75	5.00E-13	9.30E-14	5.38

Table A.6. Raw data for PBS control experiments

	TMP k_{obs} (hr^{-1})	95% CI	pH
XAD8 + Sodium Bicarbonate Buffer	0.278	0.078	7.533
	0.305	0.112	7.453
	0.350	0.031	7.333
Average	0.311		7.440
StDev	0.036		
XAD8 + PBS	0.272	0.055	7.273
	0.326	0.058	7.263
	0.250	0.080	7.250
Average	0.283		7.262
StDev	0.039		