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Sunlight-induced Photochemical Processes in Natural and Wastewater Treatment Systems

by

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This thesis entitled:

Sunlight-induced Photochemical Processes in Natural and Wastewater Treatment Systems

written by Simón Mostafa

has been approved for the
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Date: ______________

The final copy of this thesis has been examined by the signatories, and we find that both the content and the form meet acceptable presentation standards of scholarly work in the above-mentioned discipline

Abstract

Mostafa, Simón (Ph.D. Civil Engineering)

Sunlight-induced Photochemical Processes in Natural and Wastewater Treatment Systems

Thesis directed by Fernando Rosario-Ortiz, Assistant Professor, and Karl Linden, Professor, Department of Civil, Environmental and Architectural Engineering, University of Colorado at Boulder

Photochemical processes initiated by sunlight irradiation in surface waters are known to influence a variety of environmentally-relevant processes by interacting with organic matter (OM), microorganisms, and other water constituents. An important role of OM in water bodies is that of a photosensitizer in the formation of reactive intermediates (RI), including excited states, hydroxyl radicals ($HO^{\bullet}$) and singlet oxygen ($^1O_2$). Such RI are known to react at relatively high rates with various organic contaminants and microbes, thus affecting their fate in the environment as well as natural treatment systems (NTS). This work contributes to the understanding of these relevant photochemical processes by looking at both the underlying mechanisms affecting the formation of RI and the effects of such processes upon microbial populations.

Concentrations of $^1O_2$ ($[^1O_2]_{SS}$) and quantum yields (Φ_{SO}) were determined for OM present in or derived from wastewater (WWOM). Both size fractionation of WWOM and its chemical oxidation resulted in increases in Φ_{SO}. The correlations between the photosensitizing properties of WWOM and optical characteristics (e.g., absorbance, E2:E3 ratio) are evaluated and a model for the prediction of $[^1O_2]_{SS}$ is proposed.

Photophysical and photochemical processes of OM of distinct origins and molecular sizes were further evaluated through the analysis of absorbance, fluorescence, and the formation of various RI of interest. Larger size fractions displayed higher absorptivity that extends further into the visible region while smaller components displayed higher quantum yields for fluorescence and all RI measured. Possible explanations for the observed results are proposed based on the increased prevalence of non-radiative energy decay processes in larger OM molecules.

The influence of OM on the photoinactivation of *Enterococcus faecalis* was evaluated under simulated sunlight due to their relevance for disinfection in NTS. The presence OM resulted in increased inactivation rates compared to sensitizer-free experiments, suggesting that its role as a photosensitizer is greater than its impact in shielding microorganisms from light. Estimations of the endogenous and exogenous inactivation rates of *E. faecalis* as a function of depth in the presence of OM are presented and the implications of these results in the design of NTS are discussed.

A mis padres, por las raíces;

a mis hermanas, por el horizonte;

a mis sobrinos, por las estrellas.

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With love and gratitude... ¡gracias totales!

Simón

Table of Contents

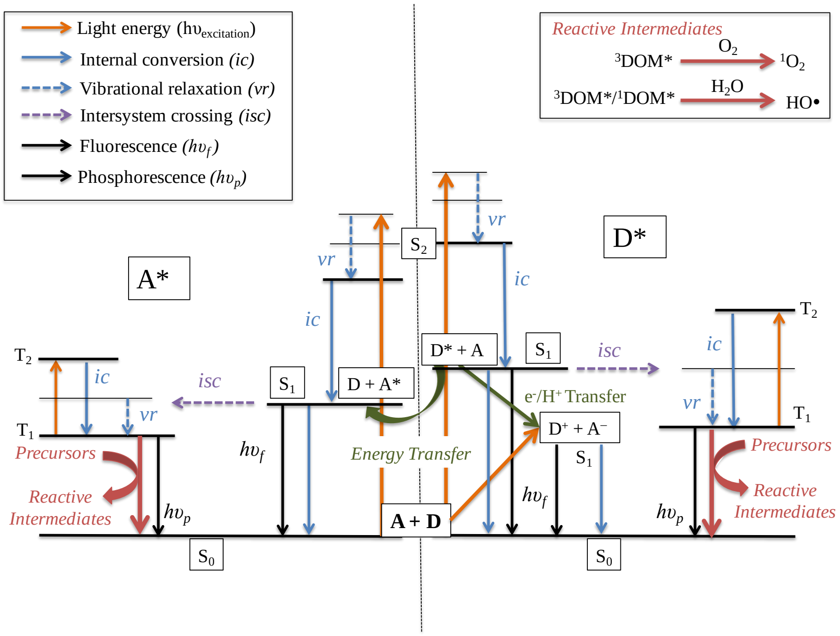
List of tables

List of figures

Chapter 1 Introduction

Research background and motivation

Organic matter (OM) of various origins is present in all water bodies and plays an important role in a number of natural processes, affecting the fate and transport of water constituents,[1–3] the depth of the photonic zone as well as the photochemical production of reactive intermediates (RI), including excited states, hydroxyl radicals ($HO^{\bullet}$) and singlet oxygen ($^1O_2$), superoxides (O_2^-), hydrogen peroxide (H_2O_2), and organic peroxyl radicals ($ROO^{\bullet}$). Such RI are known to react at relatively high rates with various organic contaminants as well as microbes.[3–11] These RI are formed in natural systems as a result of light absorption by OM. Following light-induced excitation, the excited state of an OM chromophore may return to its ground state via a radiative transition, i.e. luminescence, or undergo non-radiative processes (internal conversion, IC or intersystem crossing, ISC). ISC leads to the formation of a triplet excited state (^3OM), which can be followed by phosphorescence or $^1O_2$ formation. These processes are illustrated in Scheme 1.



Schematic 1. Conceptual illustration of photoinduced processes within organic matter. Light excitation and various possible decay processes are shown, including vibrational relaxation (vr), intersystem crossing (ISC), radiationless decay via internal conversion (IC), luminescence (hvf, hvp), energy and charge transfer from donor (D) to acceptor (A) species, as well as formation of reactive intermediates. The relative position of the various energy levels is meant for illustration purposes only and do not represent energy values of specific excited state molecules (reproduced from Chapter 3).[12]

Most of the OM found in natural systems derives from the degradation of plants and microbial processes in the water column.[3,13] On the other hand, OM present in wastewater (WWOM) contains a greater fraction of human- and microbially-derived material displaying distinct structure and chemical behaviors. This material includes N-enriched organics associated with treatment biota as well as OM displaying a lesser degree of humification and a higher fraction of hydrophilic material as compared to OM isolates from soil and natural aquatic systems.[1,14–16]

Since wastewater streams are also likely to contain higher concentrations of chemical and microbiological contaminants and enter the environment with varying degrees of treatment, they may impact ecosystems and water sources for human use. Thus, improved knowledge of relevant photochemical processes would enhance our understanding of the fate of various contaminants of concern present in surface waters, including treatment systems. While wastewater treatment is recognized to be instrumental in limiting negative health and ecological impacts, many communities around the world may not have access to infrastructure that so-called developed communities may employ for wastewater treatment. Thus, natural treatment systems (NTS) such as treatment ponds and constructed wetlands, may serve as appropriate solutions considering the need for adequate treatment of wastewater streams while understanding the economic and other limitations many communities may face.

The physical and chemical mechanisms responsible for microbial removal or inactivation within NTS include filtration, sedimentation, biocidal plant exudates, adsorption to organic matter, as well as sunlight-mediated processes. In addition, biological processes involved in microbial removal include predation, antibiotic interaction with other organisms, and natural die-off.[17–20] However, the relative contribution of different processes is not well established.

In the context of solar disinfection, most of the emphasis has traditionally been placed on the role of UV inactivation in both natural and engineered systems, due to the greater energy contained within this light range and its damaging potential on microbes. However, sunlight-mediated processes are now understood to include three mechanisms potentially leading to microbe inactivation. The first one is direct photolysis in which exposure of microbes to solar irradiation causes damage that may kill or hinder the microorganism's ability to reproduce. The second one is indirect endogenous photoinactivation, where the absorption of light by photosensitizers within the organism leads to the formation of reactive intermediates. Such RI may induce cell damage leading to microbial inactivation. A third mechanism, exogenous photoinactivation, involves photosensitizers found in the water matrix outside the cell. These photosensitizers may include natural organic matter (NOM) as well as WWOM. While the high reactivity associated with RI also leads to their relatively rapid disappearance by either physical or chemical processes, causing them to be present at relatively low concentrations in natural systems (compared to engineered and more energy-intensive systems), they can still contribute significantly to the removal of constituents of concern, including pathogens.

Previous research and relevant questions

In order to better understand the photochemistry of OM, previous works have explored how photophysical behaviors are affected by its molecular size. In this regard, differences in absorbance spectra have been reported (and partially attributed to molecular size) and characterized by increased tailing into longer wavelength regions for larger molecules.[21] Quantification of this optical phenomenon has been achieved by measuring the E4:E6 ratio,[21–23] E2:E3 ratio,[23–26] and spectral slope (S).[26–30] Early on, these observations were associated with intramolecular interactions and the formation of donor-acceptor complexes.[21,31] Such models have since been expanded, elucidating the types of species involved in the charge transfer (CT) donor-acceptor pairs.[32–34] Consistent with the increase in CT processes expected from increased molecular size, decreases in the apparent fluorescence efficiency have also been correlated to increasing OM size.[31,33,35–39] Size effects have also been linked to the formation efficiencies of various RIs in the presence of OM. For example, previous studies by our group have shown that across size fractions of wastewater-derived OM (WWOM), fluorescence quantum yields correlate to those of $HO^{\bullet}$ radicals.[40] Similar correlations to singlet oxygen formation have been reported for fluorescence of various humic and fulvic acids[41] as well as for absorbance spectral characteristics.[24–26] Furthermore, the formation of triplet states have also been reported to increase with decreasing size of OM.[37,42]

Since wastewater streams likely contain higher concentrations of chemical and microbiological contaminants, expanding our knowledge regarding the photochemical behavior of WWOM and its potential impact upon such contaminants would provide a better understanding of their fate. This has important implications when considering the role of sunlight-mediated processes in natural surface waters systems in general, but particularly those impacted by wastewater and within NTS, as demonstrated by previously published works.[8,43–47]

Sunlight-mediated processes can be of great importance within NTS where reduction of pathogens and other contaminants is a primary objective even in the absence of chemical disinfectants or any other energy- or chemically-intensive processes. Given the relatively high levels of photosensitizers within these systems (compared to un-impacted natural waters or conventionally-treated waters), indirect processes are expected to account for a greater fraction of all photochemical processes. While $HO^{\bullet}$ formation has been shown to be higher in the presence of WWOM compared to OM isolates,[48] such information is more limited with regards to $^1O_2$.[49]

In general, NTS have been shown to perform well as treatment units over a wide range of conditions leading to effluent qualities comparable to secondary treatment standards with respect to various constituents of concern.[50] Furthermore, despite the fact these systems are usually not optimized for microbial elimination, reductions in pathogen concentrations typically range between 0.5 to 3 log,[51–54] although mechanisms contributing to pathogen reduction are not fully elucidated.

Regarding the role of solar irradiation, it is hypothesized here that indirect exogenous photolysis is of significant importance as an inactivation mechanism within the type of systems discussed.[8,17,18,20] This claim is based on two phenomena taking place within these systems that would hinder UV disinfection, yet may actually enhance indirect sunlight mechanisms of inactivation. The first relates to the fact that in highly turbid and colored waters (as may be found in NTS) shorter wavelengths, i.e. UV, tend to have greater molar absorbtivities, and thus greater reductions in transmittance through the water column as compared to longer wavelength radiation, leading to a reduced impact upon microorganisms. However, the presence of organic photosensitizers (the very same species that may hinder UV transmittance) leads to an enhancement of the role of solar irradiation with regards to pathogen inactivation by allowing longer wavelength photons (including visible light) to play a role in this process via indirect exogenous photolysis.[8] The second aspect to consider is that the association of microbes to solids (which may be present in relatively high quantities) is generally assumed to lower disinfection due to a shielding effect.[55–57] However, since those solids may include the photosensitizers associated with indirect photolysis, microbes attached to such species may actually be exposed to greater RI concentrations, leading to enhanced deactivation.[58,59]

Another interesting consideration is that the presence of algae or other plants associated with these NTS increases the levels of dissolved oxygen which has been shown to enhance the photochemical processes discussed,[11,60] although debate remains in the literature regarding this claim.[8,61,62] However, enhanced photochemical degradation of organic chemicals has been reported in the presence of algae in natural freshwater environments.[7]

Several studies have been dedicated to learning more about the impact of photochemical processes upon pathogens. It has been shown that fecal coliforms are reduced to a greater extent upon exposure to sunlight in the presence of water extracted from a waste stabilization pond as compared to distilled water.[11] This research points to indirect exogenous photolysis as an explanation and to singlet oxygen and hydrogen peroxide as the main RI responsible for these results. Davies et al. have studied the deactivation of several indicator organisms exposed to sunlight within samples of a waste stabilization pond effluent.[60] It was reported that both RNA bacteriophage and Enterococci display significant susceptibility to indirect exogenous photolysis, while this is the case for E. coli and fecal coliforms only at elevated pH values and with DNA bacteriophage not being greatly affected by this phenomenon (more so by direct UV damage).[18,60] More recent studies have confirmed previous observations where MS2 virus (a RNA bacteriophage) was found to be effectively deactivated by indirect exogenous photolysis, predominantly due to the impact of singlet oxygen species.[8]

Despite significant advances in this field, several questions remain open that justify an intensified research effort. How does the source and characteristics of OM present in surface water affect the formation of reactive intermediate species, including ^{3}OM, $^1O_2$, and $HO^{\bullet}$? What does this tell us about the intramolecular processes taking place? How do they affect the inactivation of various microbial species? Can this behavior be predicted in a way that can inform the design of NTS systems or the fate of species of concern in the environment?

Based on what is known in the published literature and in an attempt to expand the current body of knowledge, a series of hypotheses were formulated and addressed through systematic research as described in the following chapters.

Goals and hypotheses

This research explores the role of sunlight-driven photochemical processes associated with dissolved OM present in wastewater and other surface waters. The relation between the structural and optical characteristics of OM and the formation of RIs that play a role in indirect sunlight-induced processes were studied. These results contribute to better estimations on systems performance based on the OM present and inform NTS design by better accounting for such photochemical processes. Three specific hypotheses were developed and explored through the research presented in this work:

I. Photochemical formation of $^1O_2$ is correlated to the optical properties and affected by molecular size and oxidation of WWOM

a) Decreasing MW leads to increased $^1O_2$ quantum yields.

b) WWOM oxidation leads to increases in $^1O_2$ quantum yields

c) $^1O_2$ quantum yields are positively correlated to the OM spectral slope and its multiplication by absorbance allows for estimations of $[^1O_2]_{SS}$

II. Quantum yields of fluorescence and RIs increase concomitantly with decreasing MW of OM due to decreasing prevalence of non-radiative transitions

III. Photoinactivation of enterococci is enhanced in NTS waters due to higher RI concentrations

a) Overall inactivation rates of *Enterococcus faecalis* increase in the presence of OM

b) The relative contribution of indirect exogenous photoinactivation increases with depth

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Chapter 2 Singlet oxygen formation from wastewater organic matter

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Abstract

Singlet oxygen ($^1O_2$) plays an important role in the inactivation of pathogens and the degradation of organic contaminants. The present study looks at the surface steady-state concentration of $^1O_2$ and quantum yields (Φ_{SO}) for organic matter present in or derived from wastewater (WWOM), including those that are partially treated and after undergoing oxidation. The surface steady state concentrations ranged from 1.23 to 1.43×10^{-13} M for bulk wastewaters under simulated sunlight. The Φ_{SO} values for these samples varied from 2.8 % to 4.7 % which was higher than the values observed for the natural organic matter isolates evaluated (1.6 – 2.1 %). Size fractionation of WWOM resulted in Φ_{SO} increases, with a value of up to 8.6 % for one of the < 1 kDa fractions. Furthermore, oxidation of WWOM by hypochlorous acid (HOCl) and molecular ozone also resulted in an increase in Φ_{SO}, with the highest measured value being 8.1 %. This research further explores the correlations between the photosensitizing properties of WWOM and optical characteristics (e.g. absorbance, E2:E3 ratio). Making use of easily measured absorbance values, a model for the prediction of $^1O_2$ steady-state concentrations is proposed.

Introduction

Organic matter (OM) in aquatic systems and its photochemical reactions are recognized to affect a number of processes of environmental significance, including serving as a source of carbon and energy as well as affecting the sequestration, transport, and chemistry of various inorganic constituents essential to ecosystems.[1–4] A particular area of interest involves the role of

OM as a naturally occurring photosensitizer, enhancing the formation of reactive oxygen species (ROS), such as hydroxyl radicals ($HO^•$) and singlet oxygen ($^1O_2$).[5] While the formation of $HO^•$ has received a great deal of attention within this context due to its ability to degrade a wide range of organic contaminants,[6–12] singlet oxygen ($^1O_2$) may also enhance the degradation of certain classes of organic compounds (e.g. furans and phenols[7]) and have a significantly higher impact upon microbes.[13–19]

Most of the OM found in natural systems derives from the degradation of plants and microbial processes in the water column.[4,6] However, OM present in wastewater (WWOM) contains a greater fraction of human- and microbially-derived material displaying distinct structure and chemical behaviors. This material includes organics associated with treatment biota as well as OM displaying a lesser degree of humification and a higher fraction of hydrophilic material as compared to OM isolates from soil and natural aquatic systems.[1,20–22] Since wastewater streams likely contain higher concentrations of chemical and microbiological contaminants, expanding our knowledge regarding the photochemical behavior of WWOM and its potential impact upon such contaminants would provide a better understanding of their fate. This has important implications when considering the role of sunlight-mediated processes in natural surface waters systems in general, but particularly those impacted by wastewater and within non-conventional wastewater treatment (NC-WWT) systems, such as treatment ponds and constructed wetlands, as demonstrated by previously published works.[14,18,23–26]

Sunlight-mediated processes can be of great importance within such NC-WWT systems where reduction of pathogens and other contaminants is a primary objective even in the absence of chemical disinfectants or any other energy- or chemically-intensive processes. Given the relatively high levels of photosensitizers within these systems (compared to un-impacted natural waters or conventionally-treated waters), indirect processes are expected to account for a greater fraction of all photochemical processes. While $HO^{\bullet}$ formation has been shown to be higher in the presence of WWOM compared to OM isolates,[27] such information is more limited with regards to $^1O_2$. Therefore, better quantification of the photosensitizing role played by OM present in wastewater (including effluent and partially treated streams) would improve our understanding of photochemical processes within natural, impacted, and treatment environments.

The formation rate of $^1O_2$ is controlled by the amount photons absorbed (related to the amount of OM present and its absorptivity) and its quantum yield (Φ_{SO}), i.e., the number of $^1O_2$ molecules formed per photon absorbed. The steady-state concentration of this species would be equal to the formation rate divided by the sum of physical quenching and reactions with other species, according to equation 1:

$$\left[{}^1O_2\right]_{SS} = \frac{k_a \Phi_{SO}[S]}{k_x[X] + k_d} \tag{1}$$

where k_a is the specific light absorbance of the water matrix (assumed to be dominated by OM), S represents a photosensitizing molecule with a singlet oxygen quantum yield of Φ_{SO}, k_x represents the reaction rate between singlet oxygen and any species X present in the water matrix (including but not limited to OM), while k_d is the rate of decay of oxygen back to its ground (triplet) state due to physical quenching without undergoing a reaction. In the case of $^1O_2$, the overall decay is dominated by physical quenching by the solvent, i.e. water ($k_{d,H2O} = 2.5 \times 10^5$ s^{-1}), and the physical or chemical quenching due to OM or other species can typically be ignored at environmentally relevant concentrations.[28] Thus, in general, higher concentrations of OM will lead to higher $^1O_2$ steady state concentrations ($[^1O_2]_{SS}$) as expected due to the role of OM predominantly as a photosensitizer but a relatively minor quencher. Not surprisingly, increasing absorbance, which is correlated to OM concentration, also relates to increasing steady state concentrations of singlet oxygen.[11] However, deviations from such correlations are observed due to differences in the efficiency of OM of different origins to photosensitize the formation of $^1O_2$, i.e. its Φ_{SO} values, which have been shown to vary by about an order of magnitude (~ 0.6 – 6 %).[11,29,30] Due to the structural complexity and diversity in the nature of chemical moieties that make up OM, it can be quite challenging to identify specific characteristics that affect photochemical behaviors. However, recent experiments point to reducible moieties, such as aromatic ketones, playing an important role in photosensitized $^1O_2$ formation.[31,32]

Different methods have been developed to measure the concentration of $^1O_2$, usually through the use of chemical probes.[33–35] However, considering the importance of $^1O_2$ in surface waters (impacted or otherwise) and natural treatment systems, methods to estimate or predict its concentration via simpler methods would be of great use. This research explores the photosensitizing properties of WWOM and the correlations between such properties and optical measurements, molecular weight (MW) fractions, and oxidation. The potential applications and limitations of models to estimate $^1O_2$ formation based on optical properties are discussed.

Materials and Methods

Sample collection/preparation. Wastewater samples were collected from two different treatment facilities, in the cities of Louisville (LWW) and Boulder (BWW) in Colorado, USA. BWW samples were collected following secondary clarification (2C) and after final chlorination (eff). LWW undergoes UV disinfection (no HOCl addition). Samples were filtered using a 0.7 µm glass fiber filter and stored at 4 ˚C minimizing light exposure until use. All experiments were conducted within one month of collection. OM isolate samples included Suwannee River natural organic matter (SRNOM), humic (SRHA) and fulvic acids (SRFA), as well as Pony Lake fulvic acid (PLFA). Isolates were obtained from the International Humic Substances Society (catalog numbers 1R101N, 2S101H, 1S101F, 1R109F). Stock solutions (50 mg/L) were prepared and diluted to a final concentration of 5 mg/L in a phosphate buffer solution (pH 7, 5 mM) before use. To evaluate the role of physical components of OM, the bulk samples were fractionated. Sample fractionation was performed using ultra-filtration (UF). Two membranes, with NMWCO of 10 kDa (Millipore) and 1 kDa (Omega), and a solvent resistant stir cell (Model XFUF 07601, Millipore, MA, USA) were used for this study. The membranes were first soaked in 1 L of Milli-Q water (resistance = 18.2 MΩ·cm), with three water changes before use. Any remaining glycerin preservative was removed by passing 200 mL of Milli-Q water through the membrane under 75 psi N_2.[36] For the oxidation experiments, chlorine was added to the filtered WW samples (LWW and BWW-2C) in the form of NaOCl to obtain final Cl concentrations of 0.07-0.56 mM. Samples were stored at room temperature until no residual HOCl was observed (minimum detection level (MDL) = 0.05 mg/L) as measured using a HACH Pocket Colorimeter (Loveland, CO), thus requiring no addition of quenching agents. O_3 stock solution (with a concentration of 0.73 mM) was added to BWW-2C samples to obtain final concentrations of 0.044 - 0.176 mM. Ozonated samples were stored for at least 24 hours before exposure experiments, and no residual O_3 is expected to be present.

Reagents and water quality analysis. Furfuryl alcohol (FFA, obtained from TCI America, Portland, OR) and methanol (ACS grade, obtained from Honeywell, Morristown, NJ) were used as received. TOC (MDL = 0.2 mg C/L) was measured using a TOC-VSCH (Shimadzu Corp., Japan) analyzer. Absorbance was measured with a Cary 100 Bio UV/Vis spectrophotometer. The E2:E3 value denotes the ratio of a sample's absorbance at 254 nm to that at 365 nm.[37] The spectral slope ($S_{275-500}$) describes the slope of a linear regression applied in the range 275-500 nm to the log-transformed absorption spectrum of each sample.[38] All absorbance measurements (and calculations derived from them) were carried out before the exposure experiments; thus, they represent the initial characteristics of the samples and do not account for potential light-induced changes over exposure time.

Singlet oxygen measurements. FFA was used as a probe to measure $[^1O_2]_{SS}$, following previously established procedures [33,39] and described here briefly. FFA was added to the samples at an initial concentration of 22.5 μM, and its concentration was monitored after exposure to solar-simulated light using liquid chromatography (Agilent, 1200 LC). Samples were placed in 2-mL clear glass vials and kept in a water bath at 20 °C during a four hour exposure to simulated sunlight. Plotting $\ln[FFA]/[FFA_0]$ versus irradiation time generates a straight line as expected from a pseudo-first order reaction rate (with $^1O_2$ at steady state). The slope obtained is divided by the second-order reaction rate constant between $^1O_2$ and FFA of 1.2×10^8 M^{-1} s^{-1} to obtain the $[^1O_2]_{SS}$.[33,40]A solar simulator (Model Sol1A, Oriel Instruments, Stratford, CT) was used for the exposure experiments, equipped either with a 450 W or 1000 W Xe lamp and 1.5 air mass filter (photon irradiance shown in Figure A.1). Methanol was also added (0.1 M) in order to quench $HO^\bullet$ which may be formed during light exposure. Failure to add a $HO^\bullet$ quencher would result in an overestimation of $^1O_2$ concentration by up to ~ 20 % (data not shown) due to a second-order reaction rate constant between FFA and $HO^\bullet$ of 1.5×10^{10} M^{-1} s^{-1}.[9,41] Measured $^1O_2$ concentrations were corrected to account for differences in the total irradiance between the simulated light and a standard sunlight spectrum (ASTM G173). $^1O_2$ quantum yields (Φ_{SO}) were calculated based on the ratio of the $^1O_2$ formation rate, R^{SO}, divided by the total number of photons absorbed, taken as the sum of the absorbance per unit time across the wavelengths of the polychromatic light source used in this study (λ = 290-400 nm). As shown in equation 1, the numerator represents the $^1O_2$ formation rate (in M s^{-1}) which can be calculated as $R^{SO} = [^1O_2]_{SS} \times k_d$. The specific light absorbance is calculated by as per equation 2[27],

$$k_a = \sum_{\lambda} \frac{E_p^0 \varepsilon_S(\lambda)\left(1 - 10^{-(\alpha(\lambda)+\varepsilon_S(\lambda)[S])z}\right)}{(\alpha(\lambda) + \varepsilon_S[S])z} \qquad 2$$

where E_p^0 is the near surface photon irradiance, ε_S is the apparent molar absorptivity, α is the unit absorbance of the background matrix, and z is the depth of the water. The specific light absorbance at each wavelength (1 nm increments) was multiplied by the concentration of OM, [S], obtained from the TOC measurements. The nitrate contribution to the total absorbance was removed by subtracting the nitrate spectrum corresponding to the concentrations found in the wastewater samples (15.8 and 4.0 mg-N/L for BWW and LWW, respectively). By summing up the resulting values, the total light absorbance of the organic matter was obtained, which represents the denominator in the quantum yield calculation. Due to the small depth of water (<1 cm) used in the solar exposure experiments, the $[^1O_2]_{SS}$ and Φ_{SO} values reported represent those found at the water surface, i.e., without significant light screening.

Results and Discussion

Water quality. Some variation was observed for pH (7.3 – 8.4) since WW samples were not buffered or pH adjusted in order to maintain the sample characteristics expected in the environment. Table 2.1 shows the water quality parameter for each of the samples studied. TOC values ranged 3.1 - 11.5 mg/L for the wastewater samples evaluated. While particles were removed from all samples using 0.7 µm filters, between 33 % and 75 % of the TOC was removed by UF during MW fractionation. E2:E3 values, $[^1O_2]_{SS}$, and Φ_{SO} are also shown in Table 2.1 and are further discussed in the following sections. The absorbance spectra for all samples are shown in Figures A.2 and A.3 of the Supporting Information.

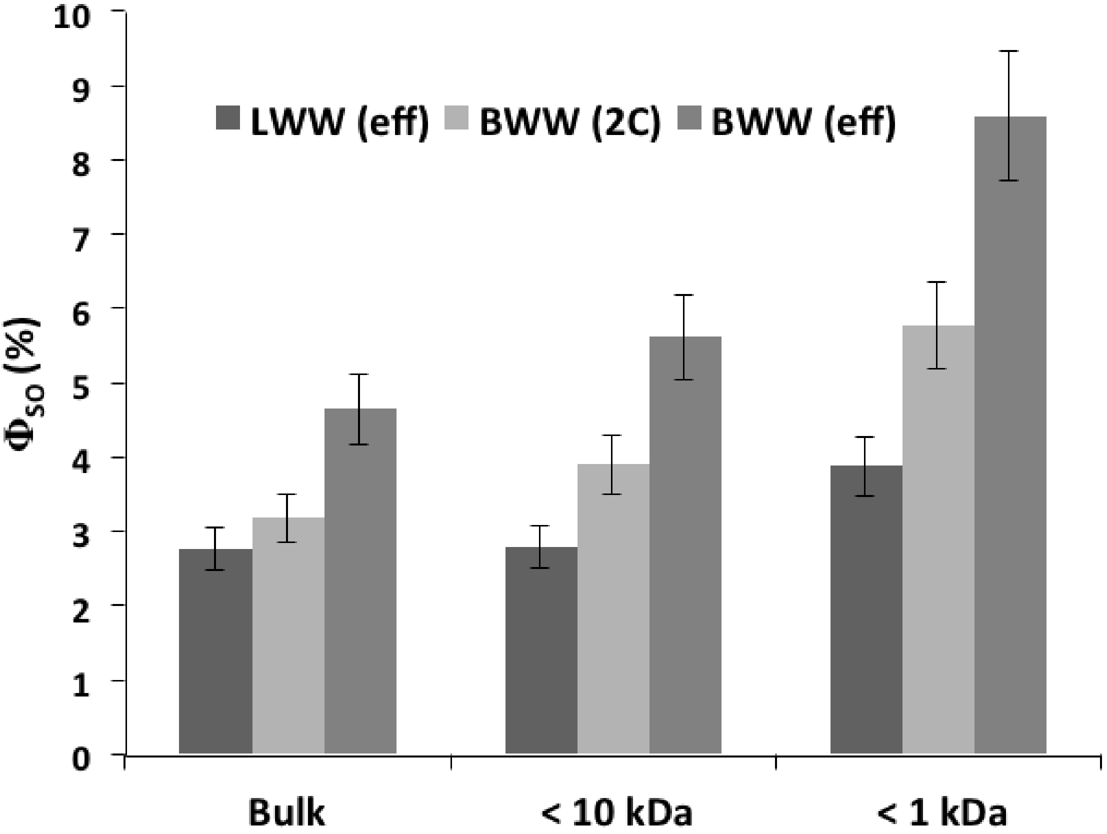
Table 2.1 Water quality parameters and $^1O_2$ formation for all samples studied. 95% confidence interval shown in parenthesis for $[^1O_2]_{SS}$ and Φ_{SO}.

Sample	TOC (mg/L)	E2:E3	$[^1O_2]_{SS} \times 10^{14}$ (M)	Φ_{SO} (%)
SRNOM	2.26	3.94	6.2 (0.69)	2.02 (0.23)
PLFA	2.09	4.50	3.9 (0.52)	2.04 (0.27)
SRFA	2.15	4.77	5.6 (0.81)	2.11 (0.31)
SRHA	2.04	3.06	9.4 (0.46)	1.60 (0.08)
LWW-eff	11.50	4.79	14.3 (1.6)	2.77 (0.31)
LWW-eff < 10kDa	6.67	4.96	11.4 (1.5)	2.79 (0.37)
LWW-eff < 1kDa	4.14	5.27	8.5 (1.3)	3.88 (0.58)
LWW + 0.14 mM Cl	11.50	6.02	15.9 (1.4)	4.53 (0.40)
LWW + 0.28 mM Cl		7.25	16.8 (1.3)	6.11 (0.49)
LWW + 0.42 mM Cl		8.52	14.9 (1.7)	6.52 (0.75)
LWW + 0.56 mM Cl		10.11	15.3 (0.89)	8.01 (0.47)
BWW-2C	8.40	4.59	12.3 (1.6)	3.18 (0.41)
BWW-2C < 10 kDa	4.64	4.86	12.0 (1.1)	3.91 (0.35)
BWW-2C < 1 kDa	3.04	5.28	11.3 (1.4)	5.78 (0.72)
BWW-eff	7.55	5.71	13.2 (1.5)	4.66 (0.54)
BWW-eff < 10 kDa	5.07	5.70	14.6 (2.3)	5.62 (0.89)
BWW-eff < 1 kDa	3.93	6.51	14.6 (2.0)	8.59 (1.19)
BWW-2C + 0.07 mM Cl	8.40	5.19	12.4 (1.3)	3.82 (0.39)
BWW-2C + 0.14 mM Cl		6.46	12.4 (1.1)	5.09 (0.43)
BWW-2C + 0.21 mM Cl		8.22	11.6 (0.88)	6.67 (0.51)
BWW-2C + 0.28 mM Cl		10.16	9.3 (0.56)	7.13 (0.43)
BWW-2C + 0.42 mM Cl		15.84	5.1 (0.40)	7.36 (0.57)
BWW-2C + 0.56 mM Cl		17.63	3.1 (0.11)	5.74 (0.21)
BWW-2C + 0.044 mM O_3		7.39	7.0 (0.72)	4.38 (0.45)
BWW-2C + 0.088 mM O_3		11.12	3.6 (0.32)	5.18 (0.47)
BWW-2C + 0.132 mM O_3		17.21	2.1 (0.19)	7.91 (0.71)
BWW-2C + 0.176 mM O_3		16.77	1.9 (0.37)	9.32 (1.82)

<u>*Singlet oxygen formation from bulk wastewaters and fractions.*</u> Table 2.1 shows the $[^1O_2]_{SS}$ and Φ_{SO} observed for a number of effluents and partially treated wastewaters, after undergoing fractionation as well as different degrees of oxidation (with HOCl and O_3). Results for the formation of $^1O_2$ in the presence of OM isolates are also presented here as a reference, allowing for a wider set of OM types to be evaluated.

In general, $^1O_2$ surface concentrations in these WW samples fall within the range of values originally measured by Haag et al. for rivers, eutrophic lakes, and WW samples.[40] However, when comparing the values presented here to others found in the more recent literature, the $^1O_2$ concentrations in WW ($1.23 \times 10^{-13} – 1.43 \times 10^{-13}$ M) tended to be higher than those found in lakes[8,11] but lower than those of rivers[11], and comparable to those found in waste stabilization ponds.[14] Thus, the impact that WW discharges may have on receiving bodies in terms of $^1O_2$ concentrations is dependent upon the characteristics of the receiving body as well as other factors, such as dilution. On the other hand, Φ_{SO} serves as a property that is independent of OM concentrations and tends to be higher for bulk WWOM samples (2.8 - 4.7 %) than the OM isolates studied here (1.6 - 2.1 %). Such Φ_{SO} values are also higher than or comparable to natural lake waters previously studied.[11,29,30]

It must be noted that due to the complexity and diversity of OM derived from different sources, broad generalizations should be avoided. OM must be understood as a broad category of molecules that will display distinct subcomponents and functional groups which affect its properties, depending on its origin and a variety of factors that may affect its fate.[4] Thus, measured behaviors for OM arise from complex interactions of such components that are difficult (and rather unpractical) to isolate. Despite such complications, trends observed at such a broad level can still provide useful information regarding the expected impacts such components



may have within water systems.

Figure 2.1Φ_{SO} for various WW size fractions. Bulk samples are filtered using 0.7 μm glass fiber filter and fractions obtained by UF. Φ_{SO} is defined as the number of $^1O_2$ molecules per photon absorbed over the 290-400 nm range under a solar simulator

The results shown in Figure 2.1 show that within each WW sample set, an increase in Φ_{SO} is observed with decreasing MW. Also, final effluent samples of BWW (after chlorination) display higher Φ_{SO} compared to second clarifier effluent, and this will be discussed in the section below in the context of oxidation. Two distinct phenomena may explain the observed trends related to varying MW. One possibility is that the lower MW fractions are intrinsically more efficient photosensitizers than the higher MW fractions, and thus the bulk samples. Another explanation is that, due to geometric effects, lower MW fractions are less susceptible to self-quenching of excited species, i.e., the process by which species generated within the OM structure are quenched before escaping to the bulk solution. The effects of self-quenching have been established[42] and given the fact that a hydrophilic probe (FFA-k_{ow} = 0.28) has been used to measure $^1O_2$ concentrations, only those molecules present in the bulk phase are accounted for. Thus, when considering the impact of size upon the concentration distribution, one would expect that excited species generated within smaller OM molecules would undergo a lesser degree of internal quenching resulting in higher (measured) concentrations and apparent formation efficiencies. It must be noted, however, that evidence of the geometric effects of OM size fractions, does not rule out the hypothesis of higher intrinsic formation efficiencies. While this topic is beyond the scope of this study, further research is currently underway to address this question. In general, these results correspond to trends previously observed when evaluating OM isolates of varying sizes (either MW-fractionated samples[43,44] or of different origins[45]), and extend such observations to WWOM, indicating that similar phenomena influence the photosensitizing efficiency of these samples.

Effect of WWOM oxidation. As shown in Figure 2.1, final effluent samples (after chlorination for BWW) display higher Φ_{SO} than the secondary clarifier effluent samples from the same treatment facility. In order to systematically evaluate this phenomenon, WWOM was oxidized by introducing two different oxidants, namely O_3 and Cl (added as NaOCl) at doses between 0.07 and 0.56 mM (with no residual present). The goal was to elucidate the changes in the optical and photosensitizing properties following such alterations. Figure 2.2 shows the changes in the absorption spectra of samples due to oxidation while Figure 2.3 shows the corresponding E2:E3 values. Upon oxidation, significant reduction of absorbance is observed, as has been previously

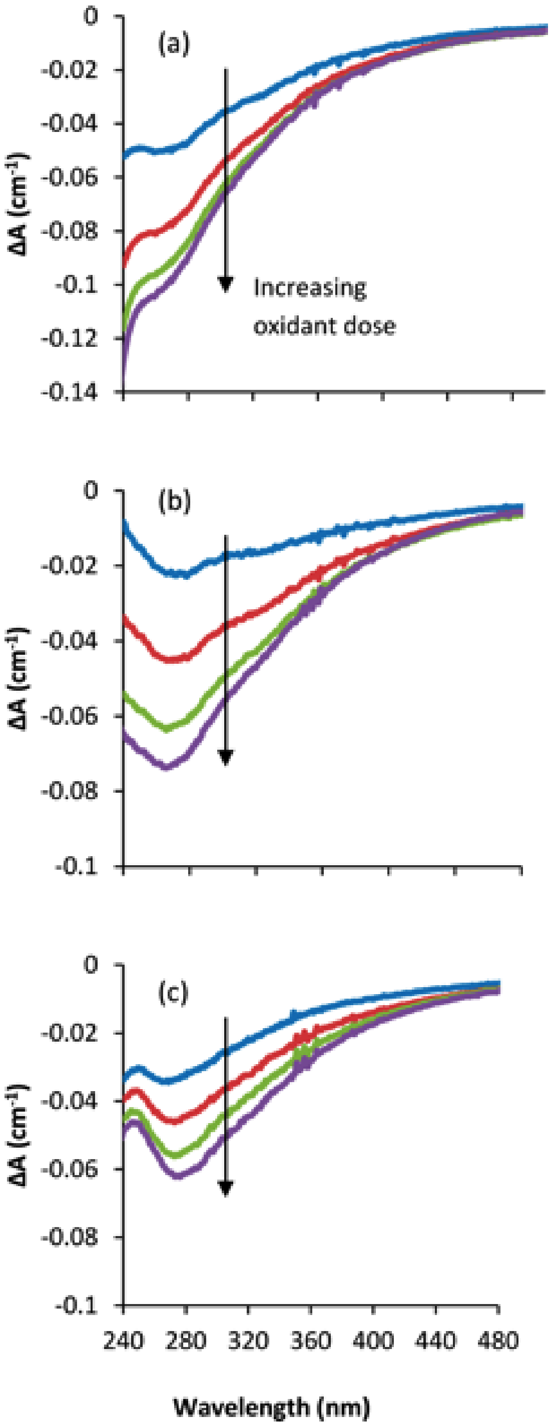


Figure 2.2 Change in absorbance for BWW (A(λ) – A_0(λ)) upon oxidation with 0.044-0.176 mM O_3 (a) and 0.14-0.56 mM HOCl (b) and for LWW with 0.14-0.56 mM HOCl (c). Samples were stored until no residual oxidant was observed before conducting final absorbance measurements. Arrows indicate the direction of increasing oxidant dose.

reported by Li et al.[46] Such changes are likely due to the incorporation into or cleavage of aromatic rings by the oxidants present, leading to a loss of light absorbing structures. It can be observed that samples oxidized with O_3 present a reduction of absorbance over a broader range of wavelengths as compared to samples oxidized with Cl, indicating that the former is a less selective oxidant. Along with a reduction in absorbance, E2:E3 values increased, as shown in Figure 2.3.

Despite the different oxidation patterns (Figure 2.2a-c) and the spread in the optical alterations represented by E2:E3 (Figure 2.3), the variation in quantum yield is very similar for all except the highest oxidant doses, as shown in Figure 2.4a. For all samples, Φ_{SO} increases upon oxidation (regardless of the oxidant used). One possible explanation for this trend is those sites which are less important in the energy transfer processes necessary for the formation of $^1O_2$ (as compared to their contribution to light absorbance) are being preferentially oxidized. However, the same may not be said about OM molecules undergoing reduction, for which quantum yields remained virtually unchanged despite absorbance loss and E2:E3 increase.[31] Another possible explanation derives from the effect that oxidation may have upon average OM molecular size, since both chlorination[47] and ozonation[48] have been shown to promote the formation of smaller OM molecules. This phenomenon would give rise to similar effects as those observed due to fractionation, namely increased E2:E3 and measured quantum yields. Furthermore, it is also possible that upon oxidation, those sites within the OM molecule more susceptible to this process would be readily oxidized. Since these may also be the most likely to react with $^1O_2$ before it escapes the photosensitizing molecule, the decreased quenching would also generate higher levels of FFA degradation, leading to higher measured $^1O_2$ concentrations and quantum yields.

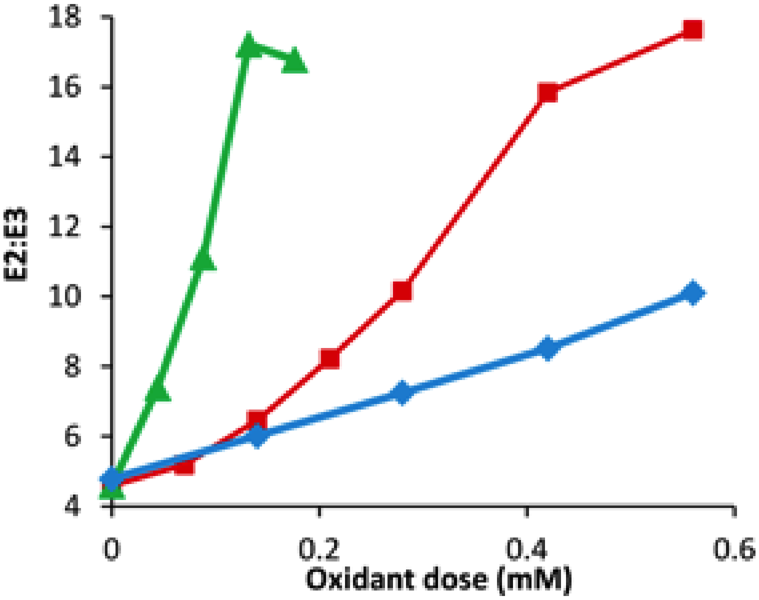


Figure 2.3 E2:E3 values (absorbance at 254 nm divided by absorbance at 365 nm) for bulk WW samples as a function of oxidant dose after addition of HOCl for BWW (■) and LWW (♦) and of O_3 for BWW (▲).

Finally, it is possible that the changes in the trends of Φ_{SO} at the highest levels of oxidation (either decrease or flattening) is caused by significant changes to the WWOM sites which affect its photosensitizing properties, but had not been preferentially oxidized at lower doses. These trends can also be explained by considering that the different mechanisms explained above may be more or less relevant as function of oxidant dose. For example, disaggregation may play a greater role at lower oxidant concentrations, whereas destruction of chromophores could be more prevalent at the higher doses with less significant increases in quantum yields.

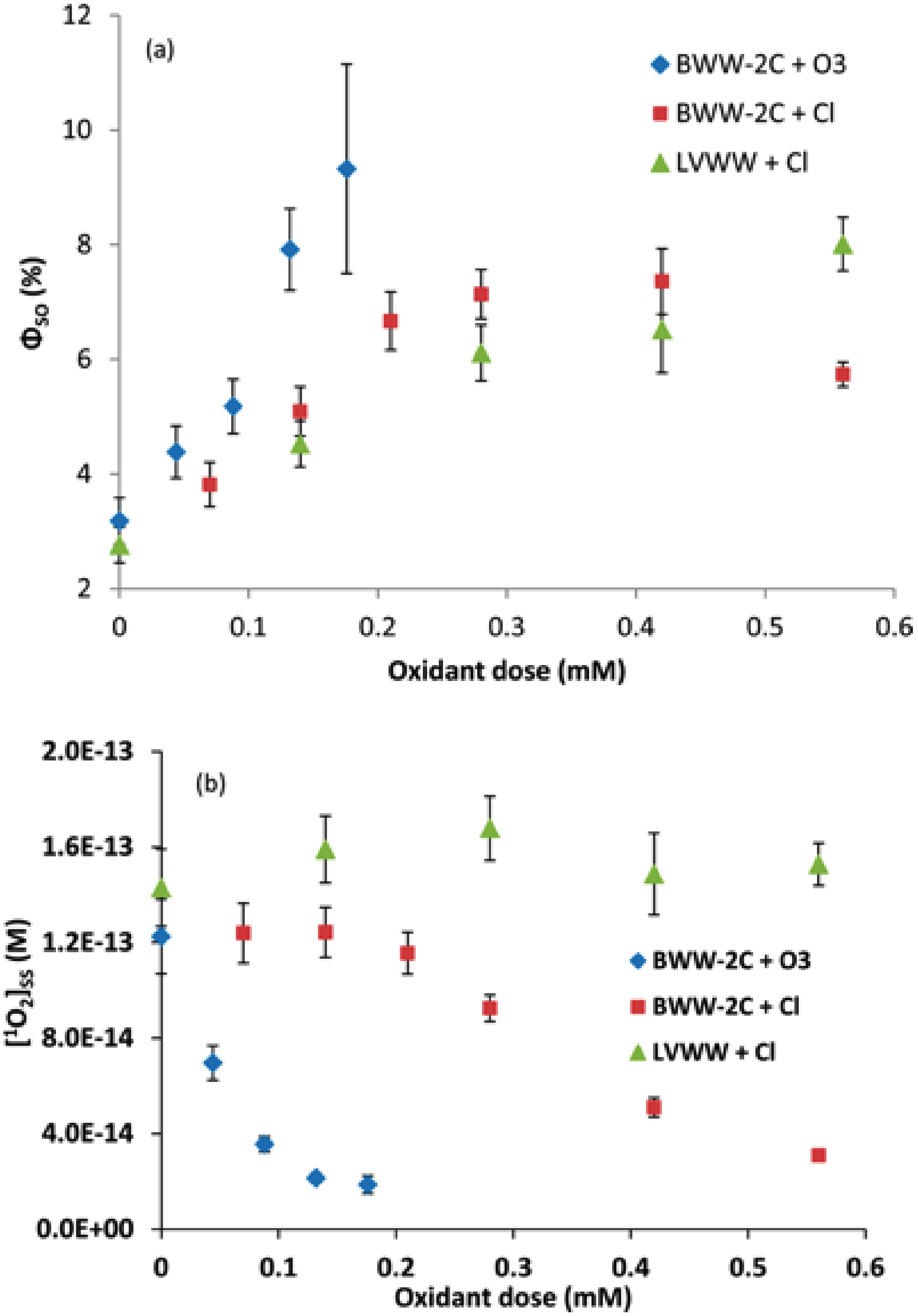


Figure 2.4 Φ_{SO} (a) and surface $^1O_2$ steady-state concentration (b) as a function of oxidant dose with addition of HOCl for BWW (■) and LWW (▲) and of O_3 for BWW (♦), with no residual oxidant present at the time of irradiation experiment. Similar increases in the Φ_{SO} are observed for all samples, but the impact upon $[^1O_2]_{SS}$ is more difficult to predict, due to simultaneous increase in Φ_{SO} and decrease in absorbance.

Given the decrease in absorption and the increase in quantum yields upon oxidation, the net effect upon singlet oxygen formation rates (and thus steady-state concentrations) is difficult to predict as illustrated in Figure 2.4b, where the overall change in $[^1O_2]_{SS}$ varies considerably from one sample to another. While $[^1O_2]_{SS}$ appears to remain relatively unchanged or to decrease with oxidation in the small depth represented by our samples, the overall formation through the depth of the water column is likely to increase with the increasing Φ_{SO} values.

Relation between optical and photosensitizing properties. Certain OM characteristics influence optical properties and photosensitizing efficiency, causing these parameters to vary simultaneously. Such co-variation, originating primarily from structural and electronic behavior characteristics within the OM molecules, can become a useful tool for assessing certain photochemical properties. Thus, correlations emerge as shown in Figure 2.5 where Φ_{SO} tend to increase with increasing E2:E3 values (except for highly oxidized samples, as discussed below). In the case of E2:E3, both geometric and charge transfer (CT) effects may influence the (measured) formation rates of singlet oxygen. According to the model proposed by Sharpless[31] the co-variation between E2:E3 and quantum yields relates to the CT behavior of OM molecules. This model suggests those species that participate in CT processes are less likely to undergo intersystem crossing necessary for the formation of the OM triplet and subsequent energy transfer to molecular oxygen. Thus, an increase in CT absorption (and associated decrease in E2:E3) corresponds to lower observed photosensitizing efficiency. As discussed above, the origin of such correlation could just as well be explained by variations in CT behavior for different types of OM or by geometric effects, yet such explanations are not mutually exclusive. In the latter case, the increase in E2:E3 associated with decreasing particle size (caused by either fractionation or oxidation) related to intra-molecular interactions,[49,50] provides a suitable explanation for the observed results.

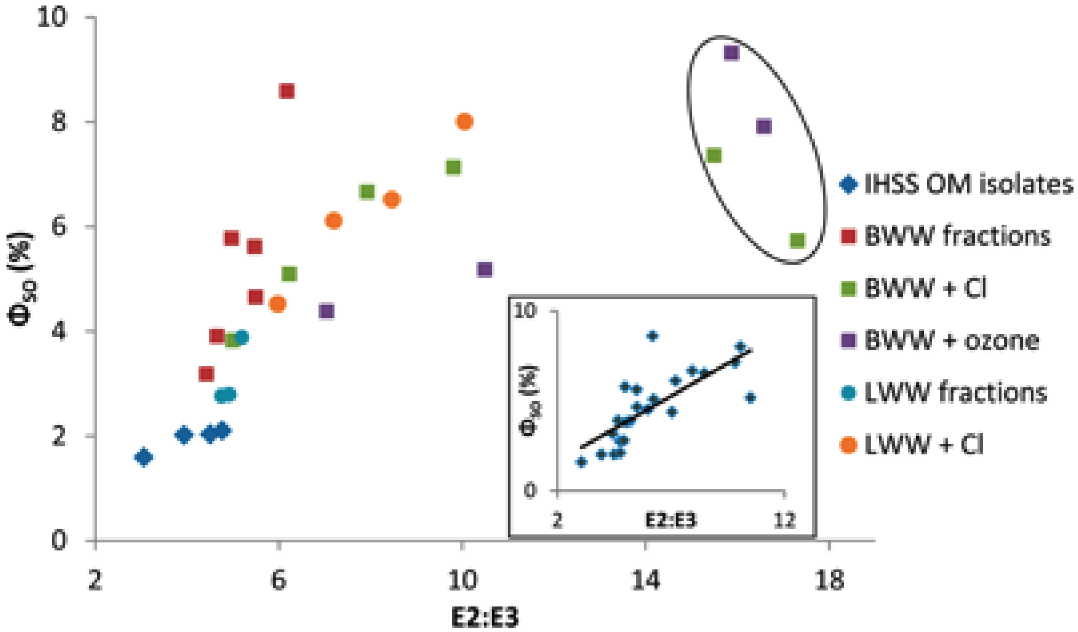


Figure 2.5 Φ_{SO} for all samples plotted against E2:E3. Circled samples are those BWW samples exposed to the highest oxidant doses. Inset: Plot of all samples except those circled in the main figure, showing correlation between quantum yield (Φ_{SO}) and absorbance spectrum characteristics (E2:E3). Fit is a linear regression ($\Phi_{SO} = 0.72 \times (E2{:}E3) + 0.20$, P-value = 5.3×10^{-5}) with slope falling within range of values previously reported in the literature.

The Φ_{SO} vs. E2:E3 correlation, however, becomes weaker when the WWOM undergoes a certain degree of oxidation as shown in Figure 2.5. The data points contained within the circle are those BWW samples that underwent the highest oxidation. However, if these samples are excluded from the plot (as shown in the inset), a trend appears that corresponds well with previously published data sets which correlate quantum yields to E2:E3. The fitting line displays a slope of 0.72 (P-value = 5.3×10^{-5}), which falls within the relatively wide range of reported values (0.196-0.871).[11,30]

These results, in conjunction with previous reports, indicate that while there is a correlation between optical properties and photosensitizing behavior, such correlations should be used with caution, especially as a broader set of OM sources are considered. In this case, processes such as abiotic oxidation appear to alter the optical properties to a degree where the established correlations no longer apply. This points to the explanation stated above, where properties such as size and CT mechanisms lead to co-variation of optical and apparent photochemical properties, yet cannot fully account for other types of chemical alterations. Furthermore, the presence of other WW constituents, e.g. nitrate, may alter the optical properties in ways not accounted for in the preceding discussion. Subtraction of the nitrate contribution to light absorbance did not significantly alter the results presented here (as shown in Figure A.4); however, it may play a greater role under different conditions.

Steady-state concentration modeling. The formation rate of singlet oxygen is the product of the number of photons absorbed by OM and its Φ_{SO} values and, as discussed above, it is proportional to $[^1O_2]_{SS}$. Thus, it follows that the multiplication of parameters that serve as surrogates to those properties would allow for the $^1O_2$ surface concentrations to be estimated making use of relatively simple methods, e.g. absorbance measurements. An example of such correlation is shown in Figure 2.6, where proportionality can be established between steady-state concentrations and the product of two optical properties, one positively correlated to quantum yield and another to total absorbance. Figure 2.6a shows $[^1O_2]_{SS}$ plotted against the product of the absorbance at 300 nm (related to total absorbance) and the spectral slope, $S_{275-500}$ (related to photosensitizing efficiency). On the other hand, in Figure 2.6b $[^1O_2]_{SS}$ is plotted against the product of E2:E3 and the absorbance at 365 nm, or simply the absorbance at 254 nm. In both cases, all measured values fall within a factor of two from the predicted values. It must be noted that the fit in Figure 2.6b is not an indication of simple proportionality where higher absorbance leads to higher $^1O_2$ production since this fit is better than those obtained by plotting $[^1O_2]_{SS}$ vs.

absorbance at 300 nm or 365 nm (not shown). Furthermore, wavelengths < 290 nm do not actually reach the sample since they are blocked by the glass vial used during the exposure.

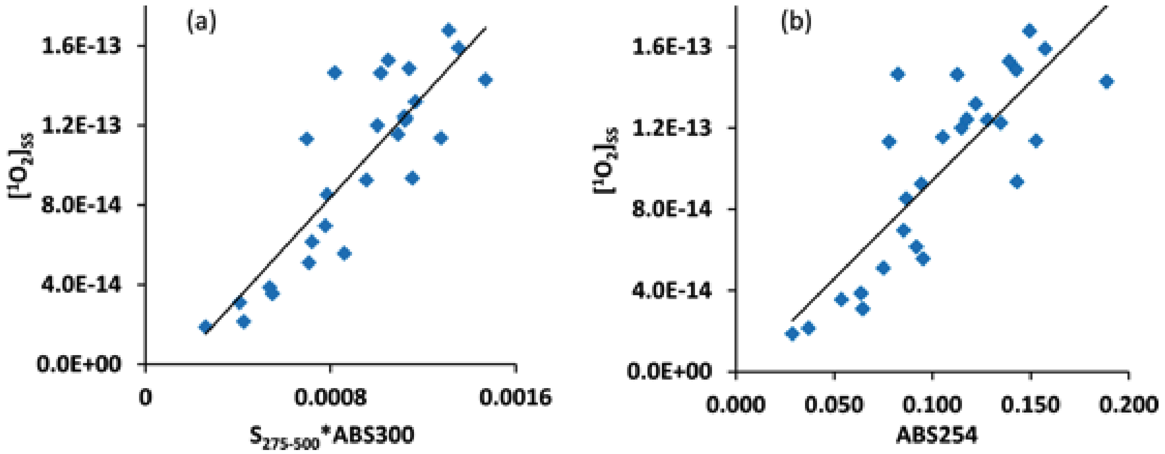


Figure 2.6 [$^1O_2$]$_{SS}$ plotted against [$S_{275\text{-}500}$ x ABS300] (a) and ABS254 (or E2:E3 × ABS365) (b), with linear regressions. The equations associated with these fits are ($S_{275\text{-}500}$ × ABS300)·(1.3×10^{-10}) – 1.8×10^{-14} for the first and (ABS254)·(9.7×10^{-13}) – 2.7×10^{-15} for the second (P-values are 1.1×10^{-8} and 1.8×10^{-7}, respectively).

Environmental implications

The results presented here point to important trends with regards to the photochemical behavior of OM present in wastewater streams. In cases where the main concern lies in the role of $^1O_2$ in its environment, bulk steady state concentrations represent an appropriate measure of the concentrations that are expected to be present within the water column. This phenomenon may have important implications in treatment systems, where association with particles leads to the shielding of solar UV light which may substantially reduce the impact of direct photolysis processes.[51–53] However, hydrophobic contaminants or particle-associated microbes may encounter significantly higher concentrations since they would tend to be found closer to the singlet oxygen source.[6,13,42]

Knowledge of $^1O_2$ formation rates (and further improvements of predictive models) helps to better understand the role of ROS within WW streams and the potential impacts of such streams upon receiving water bodies. However, due to the complexity and variability of OM molecules and of the composition of WW streams, caution should be used when attempting to predict the photochemical behavior of OM based on surrogate optical properties. Models should only be used to establish expected qualitative trends rather than as quantitative predictive models. Overall, the quantum yields of WWOM tend to be comparable or higher than those of OM standard isolates and natural water samples, and increase with chemical oxidation, e.g., chlorination during treatment. While variations in steady-state concentrations are likely be more dependent upon factors such as degree of dilution, in general, WW-impacted bodies are expected to display higher concentration of $^1O_2$ than un-impacted ones and more so for WW streams that undergo chemical oxidation.

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Supporting Information Available

Solar simulator photon irradiance, absorbance spectra for all samples, and $[^1O_2]_{SS}$ correlation to optical properties (after nitrate absorbance subtraction) are shown in Appendix A: Supplemental Information.

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Chapter 3 Relation between optical properties and formation of reactive intermediates from different size fractions of organic matter

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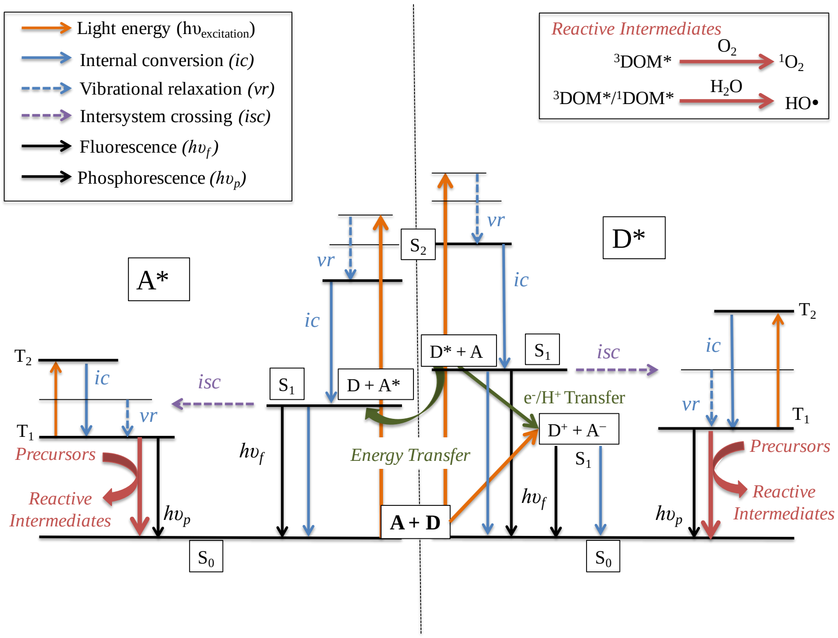
Abstract

The photophysics and photochemistry of aquatic organic matter (OM) are of interest due to their role in defining the reactivity of this material in the environment. Studies have demonstrated that molecular size and composition are important determinants of the photochemical behavior of OM, including the formation of reactive intermediates (RIs). The present study expands on previous works by evaluating both photophysical and photochemical processes of OM of distinct origins and molecular sizes through the analysis of absorbance and fluorescence along with the formation of various RIs of interest (^{3}OM, HO$^\bullet$, $^1O_2$). In general, larger size fractions displayed higher absorptivity that extends further into the visible region. On the other hand, smaller components displayed higher quantum yields for fluorescence and all RIs measured. In accordance with hypotheses previously formulated, possible explanations for the observed results are proposed based on the increased prevalence of non-radiative energy decay processes in larger OM molecules.

Introduction

Organic matter (OM) of various origins is present in all water bodies and plays an important role in a number of natural processes, affecting the fate and transport of water constituents,[1–3] light absorption and the depth of the photic zone,[4,5] as well as the photochemical production of reactive intermediates (RIs),[6–9] including excited states, hydroxyl radicals (HO$^\bullet$)

and singlet oxygen ($^{1}O_2$). RIs are known to react at relatively high rates with various organic contaminants as well as microbes.[3,10–17] Following light-induced excitation, the excited state of an OM chromophore may return to its ground state via a radiative transition, i.e. luminescence, or undergo non-radiative processes (internal conversion, IC or intersystem crossing, ISC). ISC leads to the formation of a triplet excited state (3OM), which can be followed by phosphorescence or $^{1}O_2$ formation. These processes are illustrated in Scheme 1.



Scheme 1. Conceptual illustration of photoinduced processes within organic matter. Light excitation and various possible decay processes are shown, including vibrational relaxation (VR), intersystem crossing (ISC), radiationless decay via internal conversion (IC), luminescence (hvf, hvp), energy and charge transfer from donor (D) to acceptor (A) species, as well as formation of reactive intermediates. The relative position of the various energy levels is meant for illustration purposes only and does not represent energy values of specific excited state molecules.

The photochemistry and photophysics of OM are of interest to environmental scientists and engineers; yet, due to its molecular complexity, it is difficult to isolate and study individual compounds and their behavior. Instead, measuring bulk characteristics and relevant processes offers an insight into the overall properties of the material. The relative efficiency at which each of these processes occurs is measured by its quantum yield (Φ), which is defined as the number of occurrences of a given process per photon absorbed.[18]

Previous works have explored how photophysical behavior of OM is affected by its molecular size. In this regard, differences in absorbance spectra have been reported (and partially attributed to molecular size) and characterized by increased tailing into longer wavelength regions for larger molecules.[19] Quantification of this optical phenomenon has been achieved by measuring the E4:E6 ratio,[19–21] E2:E3 ratio,[21–24] and spectral slope (S).[24–28] Early on, these observations were associated with intramolecular interactions and the formation of donor-acceptor complexes.[19,29] Such models have since been expanded, elucidating the types of species involved in the charge transfer (CT) donor-acceptor pairs.[30–32] Consistent with the increase in CT processes expected from increased molecular size, decreases in the apparent fluorescence efficiency have been correlated to increasing OM size.[29,31,33–37] Size effects have also been linked to the formation efficiencies of various RIs in the presence of OM. For example, previous studies by our group have shown that across size fractions of wastewater-derived OM (WWOM), fluorescence quantum yields correlate to those of HO• radicals[38] while E2:E3 ratios are positively correlated to singlet oxygen quantum yields.[28] Similar correlations to singlet oxygen formation have been reported for fluorescence of various humic and fulvic acids[39] as well as for absorbance spectral characteristics.[22–24] Furthermore, the formation of triplet states have also been reported to increase with decreasing size of OM.[35,40]

In this contribution, we expand on the work of our group and others by assessing the formation rates of various RIs and conducting detailed optical measurements simultaneously on various OM size fractions. Evaluating the behavior of OM samples with very different compositions (Suwannee River natural organic matter and WWOM) that still display similar trends as a function of size provides a more complete picture of the photophysics of these molecules and of the underlying mechanisms. These results are combined with previously proposed mechanisms to put forward an explanation of OM photophysical and photochemical behaviors based on relevant intramolecular processes. It is proposed here that increasing molecular size of OM leads to an increase in the prevalence of non-radiative decay relative to other processes, such as luminescence and the formation RIs.

Materials and methods

Sample collection and preparation. Two different sets of samples were used for this study representing OM of different origins, namely Suwannee River natural organic matter (SRNOM) and treated wastewater effluent (BWW). SRNOM isolate was obtained from the International Humic Substances Society (catalog number 1R101N). After dissolving the SRNOM, the stock solution was diluted to a final concentration of 8.8 mgC/L in a phosphate buffer solution (10 mM, pH 7.2). Wastewater effluent was obtained from the Boulder Wastewater Treatment Facility after undergoing complete treatment (no chemical disinfection, e.g. chlorination, was performed). Within 8 hours of collection, samples were filtered through a 0.7 μm glass fiber filters that were muffled at 550 °C for 3 hours and rinsed. Filtered samples were kept refrigerated at a temperature of 4 °C in the dark until further use.

Each source water was fractionated using ultrafiltration membranes into three size fractions: less than 1 kDa, 1-10 kDa, and greater than 10 kDa. To achieve this, the OM with MW < 1 kDa was separated in a Millipore tangential flow ultrafiltration system with an Amicon regenerated cellulose cartridge with a nominal molecular weight cut-off (NMWCO) of 1 kDa. The retentate was further fractionated in a solvent-resistant stir cell (Model XFUF 07601, Millipore, MA, USA) using a regenerated cellulose membrane with a NMWCO of 10 kDa (Millipore), resulting in a fraction with MW > 10 kDa (retentate) and an intermediate fraction with 1 kDa < MW < 10 kDa (permeate). Initially, retentate solutions were diluted by three times the initial volume with the phosphate buffer solution and then filtered so that the initial volume remained in the retentate. In an attempt to remove OM from the retentate that was less than the NMWCO, the retentate from each fractionation step was rinsed by diluting in 10 mM phosphate buffer and filtering again. The rinsing steps were repeated until there was a less than 5% change in the permeate absorbance at 254 nm (~ 4-5 rinses), but likely did not remove all the DOM that could potentially pass through the membrane. Concentrations of Cl- and Br- in the WW samples were measured by ICP-MS. All WW samples were adjusted to the same concentration of these ions (72.5 mg/L for Cl- and 0.3 mg/L for Br-) by addition of NaCl and KBr to allow for an accurate inter-comparison of fractions considering the impact of halides on the formation of RIs.[41]

Characterization and optical measurements. Dissolved organic carbon (DOC) measurements were conducted using a TOC-VCSH (Shimadzu Corp., Japan) analyzer while nitrite (NO2-) and nitrate (NO3-) were measured using FIA-IC (Lachat QuikChem 8500 Flow Injection Analyzer).

Size exclusion chromatography (SEC) was performed using an Agilent 1200 series high performance liquid chromatograph with a Waters Protein-PakTM 125 7.8 x 300 mm column (Milfrod, MA). The detector was an Agilent diode array that monitored UV absorbance at a wavelength of 254 nm. The mobile phase buffer consisted of 0.0024 M NaH2PO4, 0.0016 M Na2HPO4, and 0.025 M NaSO4, and the flow rate was 0.7 ml/min.

For the optical measurements, the concentrated fractions were diluted with 10 mM phosphate buffer to an absorbance less than 0.05 at 350 nm. UV-Vis absorption was measured in triplicate from 200 to 600 nm in 1 nm increments in a 1 cm pathlength quartz cuvette (Cary 100, Agilent Technologies CA). The E2:E3 value denotes the ratio of absorbance at 254 nm and 365 nm. Absorption coefficient, a(λ), was calculated according to equation 1, where A is the absorbance at wavelength λ in nanometers, and L is the path length in centimeters. The spectral slope (S) was determined by fitting the non-linear regression to the absorption coefficients from 300 to 600 nm following equation 2, where λr is the reference wavelength (350 nm).[27,42]

$$a(\lambda)=2.303A(\lambda)/L \qquad (1)$$

$$a(\lambda)=a(\lambda r)\ e^{-S(\lambda-\lambda r)} \qquad (2)$$

Fluorescence excitation-emission matrices (EEMs) were measured in triplicate for each sample (Fluoromax-4, John Yvon Horiba, NJ). Excitation wavelengths ranged from 240 to 450 nm in 5 nm increments, and the emission wavelengths ranged from 300 to 560 nm in 2 nm increments. Both the excitation and emission bandpasses were set to 4 nm, and the integration time was 0.25 s. Fluorescence intensities were measured in ratio mode (signal divided by reference). Lamp scans, cuvette contamination checks and Raman scans were performed daily to verify calibration. Fluorescence data were corrected for inner filter effects, normalized to the area under Raman peak for 18 MΩ lab-grade water at 370 nm excitation and blank subtracted, according to published methods.[43]

The fluorescence quantum yields were determined for each sample relative to a 1 mg/L quinine sulfate standard in 0.1N H2SO4 following previously published methods except that the quinine sulfate absorbance and fluorescence were measured at the same excitation wavelength as the DOM sample.[44–46] Quantum yields were calculated as a function of excitation wavelength from 290 to 380 nm. The calculation assumes a quinine sulfate quantum yield of 0.51, and the uncertainty reported is based on the error propagation of both fluorescence and absorption measurements between triplicates. The fluorescence index (FI) is reported for each fraction as a measure of compositional differences. FI is defined as the ratio of emission intensities at 470 and 520 nm at an excitation wavelength of 370 nm.[47,48]

Reactive species measurements. Furfuryl alcohol (FFA), benzene, and 2,4,6-trimethylphenol (TMP) were used as probes for reactive intermediates $^1O_2$, HO$^•$, and ^{3}OM, respectively.[28,49] FFA was added to the samples at an initial concentration of 22.5 μM along with methanol (0.1 M) in order to quench HO•, which may be formed during light exposure. For the HO• experiments, benzene (99.8% Alpha Aesar, MA, USA) was added at a concentration of 3 mM, causing it to be the main HO• scavenger in the system[49] and leading to the formation of phenol. Bovine liver catalase (Sigma-Aldrich, MO, USA) was added at a concentration of 20 unit/mL to prevent the formation of hydrogen peroxide, thus preventing HO• formation via photo-Fenton processes. TMP was used as a probe to assess the formation of OM excited triplet states,[35,50–52] by measuring its degradation from an initial concentration of 3.67 μmol/L.

All samples were placed in 2-mL clear glass vials and kept in a water bath at 20 °C during exposure to simulated sunlight. A solar simulator (Model Sol1A, Oriel Instruments, Stratford, CT) was used for the exposure experiments, equipped with a 1000 W Xe lamp and 1.5 air mass filter. Probe concentrations were monitored after light exposure using liquid chromatography (Agilent, 1200 LC). Quantum yields for $^1O_2$ (Φ_{SO}), HO$^•$ (Φ_{HO}), and TMP degradation (Φ_{TMP}, indicative ^{3}OM formation) were calculated based on the ratio of the reactive species formed ($^1O_2$, HO$^•$) or TMP degraded, divided by the total number of photons absorbed per unit time over the wavelength range of the polychromatic light source used in this study (λ = 290-400 nm). The contribution of nitrate to the formation of HO• was factored out for the calculation of ΦHO. Details of the methodology used can be found in previous publications,[28,49] except for TMP degradation experiments. In this case, analysis was similar to that of FFA degradation, where pseudo-first order kinetics are assumed and the degradation rate, k_{TMP}, represents the slope of the linear regression curve of $\ln[TMP]/[TMP]_0$ versus irradiation time.

Results and discussion

Water quality and molecular size. The results of the water quality analyses, including pH and DOC concentration are shown in Tables 3.1 and 3.2 for SRNOM and BWW, respectively, along with optical parameters including spectral slope, E2:E3 ratio, and specific absorbance at 254 and 350 nm. Figure 3.1 displays the results of the SEC measurements normalized to the total area. The weighted average of the elution time (t_{SEC}) of the chromophoric dissolved OM, as calculated from the SEC measurements, is shown in Tables 3.1 and 3.2 with increasing times corresponding to decreasing OM size. While each fraction has a different tSEC,, there is overlap between the specta due to the heterogeneity of DOM. Ultrafiltration membranes are typically designed to retain 90% of a protein solution near the reported NMWCO. Considering that DOM is chemically different from the proteins used by the manufacturer for size calibration, it is expected that a portion of DOM greater than the NMWCO will still pass through the filters. As a result, some of the OM molecules will reside outside of the stated NMWCOs following ultrafiltration.[53]

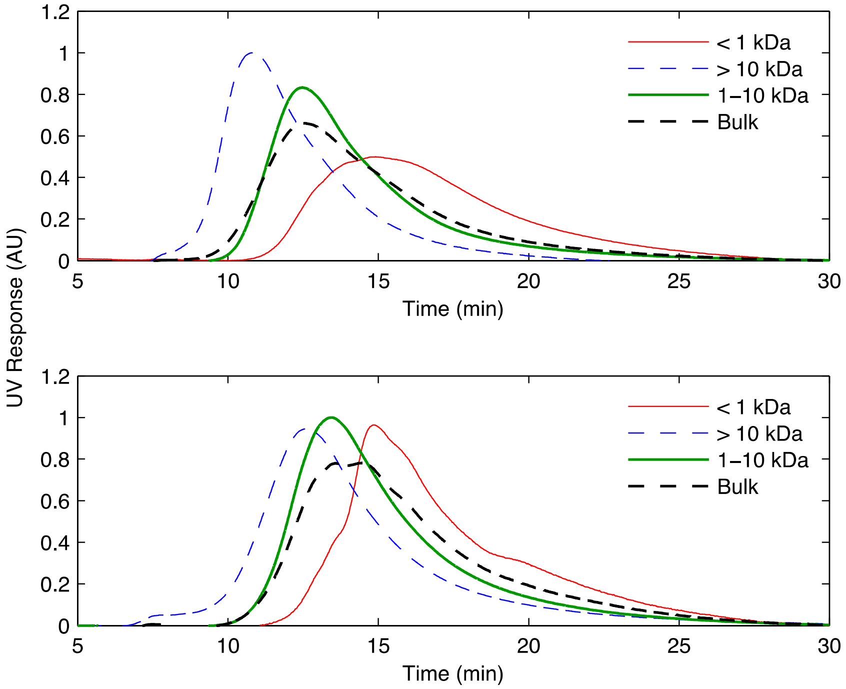


Figure 3.1 Size exclusion chromatograms with UV$_{254}$ detection for SRNOM and BWW size fractions.

Optical properties. Absorbance spectra for all the samples studied are presented in Figure 3.2 and display typical featureless curves of decreasing absorbance with increasing wavelength.

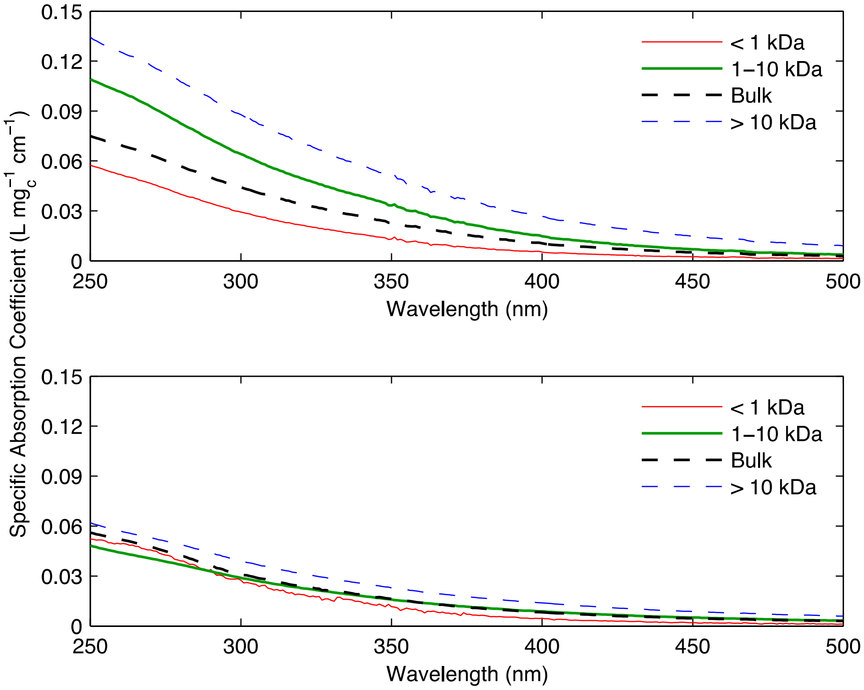


Figure 3.2 Specific absorption spectra for each size fraction. Absorption coefficients calculated using equation 1 are normalized to the sample DOC concentration.

For both OM sources, smaller molecular size fractions had higher spectral slope values (0.024 and 0.017 for the <1 kDa fraction of BWW and SRNOM, respectively) compared to the bulk, unfractionated samples (~ 0.014 for both), as shown in the tables below. Furthermore, specific absorbance at 254 and 350 nm generally increased with increasing size, in agreement with previous reports,[53,54] except for the specific absorbance at 254 nm being higher for the smallest than for the mid fraction in BWW. These results could be explained in part by differences in composition across the various fractions, with increasing concentration of phenolic groups in the larger size fractions as has been reported, for example, in the case of Laurentian fulvic acid.[29]

Table 3.1 SRNOM samples characteristics and quantum efficiencies of various processes. The standard deviation between analytical triplicates is listed in parentheses.

Sample	*SRNOM*			
Fraction	*Bulk*	*> 10 kDa*	*1-10 kDa*	*< 1 kDa*
pH	7.2	7.2	7.2	7.2
DOC (mg C/L)[a]	4.9	1.9	3.5	3.0
$SUVA_{254}$ *(L/mg C-m)*	3.16	5.65	4.60	2.40
$SUVA_{350}$ *(L/mg C-m)*	0.99	2.17	1.44	0.56
S	0.014	0.012	0.014	0.017
E2:E3	4.11	3.25	4.18	5.83
FI	1.29	1.06	1.19	1.47
t_{SEC} *(min)*	14.8	12.4	14.0	16.1
Φ_{FL} *(%)*	0.007 (0.00017)	0.001 (0.00004)	0.005 (0.00014)	0.023 (0.00063)
Φ_{SO} *(%)*	1.85 (0.15)	0.44 (0.05)	1.44 (0.11)	4.39 (0.33)
Φ_{HO} *(%)*	0.0041 (0.0020)	0.0035 (0.0013)	0.0028 (0.0008)	0.0068 (0.0015)
Φ_{TMP} *(%)*	0.0131 (0.0009)	0.0049 (0.0003)	0.0062 (0.0005)	0.0284 (0.0029)

[a] The DOC values for the size fractions do not add up to the bulk concentration since volume of each fraction was different.

Table 3.2 BWW samples characteristics and quantum efficiencies of various processes. The standard deviation between analytical triplicates is listed in parentheses.

Sample	*BWW*			
Fraction	*Bulk*	*> 10 kDa*	*1-10 kDa*	*< 1 kDa*
pH	7.4	7.3	7.2	7.7
DOC (mg C/L)[a]	5.0	10.0	11.7	1.5
$SUVA_{254}$ *(L/mg C-m)*	2.43	2.59	2.01	2.48
$SUVA_{350}$ *(L/mg C-m)*	0.70	0.99	0.69	0.50
S	0.014	0.010	0.012	0.024
E2:E3	4.62	3.19	3.70	7.80
FI	2.05	1.51	1.54	2.14
t_{SEC} *(min)*	16.0	13.9	15.2	17.8
Φ_{FL} *(%)*	0.027 (0.00102)	0.004 (0.00015)	0.008 (0.00037)	0.048 (0.00190)
Φ_{SO} *(%)*	3.29 (0.63)	1.15 (0.07)	1.91 (0.16)	6.18 (0.64)
Φ_{HO} *(%)*	0.0032 (0.0005)	0.0018 (0.0003)	0.0016 (0.0001)	0.0497 (0.0111)
Φ_{TMP} *(%)*	0.0435 (0.0034)	0.0014 (0.0001)	0.0027 (0.0002)	0.1327 (0.0130)

[a] The DOC values for the size fractions do not add up to the bulk concentration since volume of each fraction was different.

The fluorescence EEMs for all samples are shown in Figures 3.3 and 3.4. Since the DOC concentration of each fraction was different, the intensities were normalized per unit carbon to be able to directly compare them. On a per unit carbon basis, the smallest molecular size fraction has the highest intensities compared to the large size fractions. For SRNOM, the bulk sample intensities fall closest to the the 1-10 kDa fraction whereas the bulk BWW sample intensity falls between those of the <1 kDa and 1-10 kDa fractions.

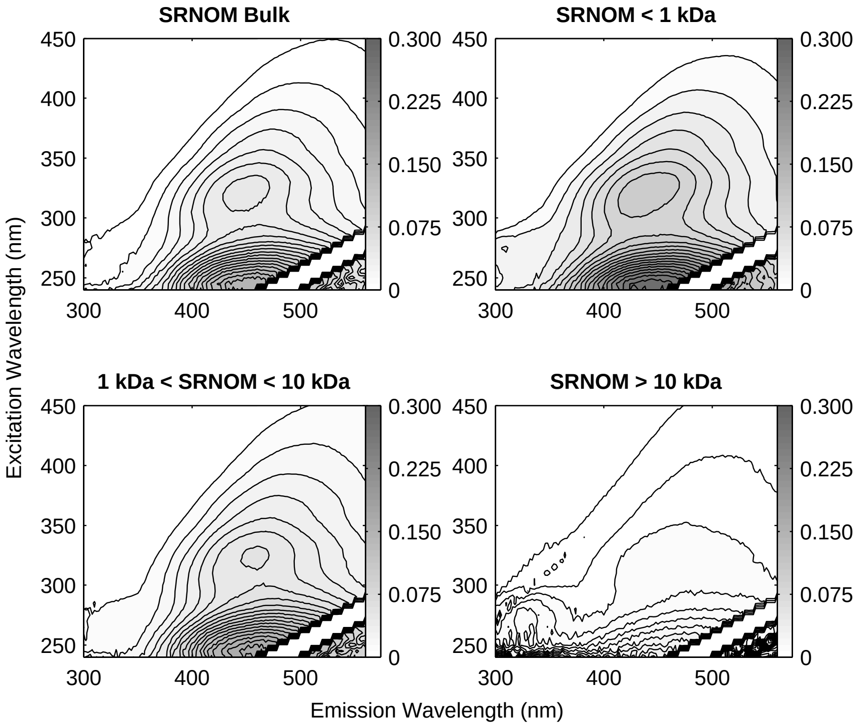


Figure 3.3 Fluorescence EEMs for SRNOM and each size fraction. Each EEM is the average EEM from three replicates. The intensities have been normalized to the DOC concentration.

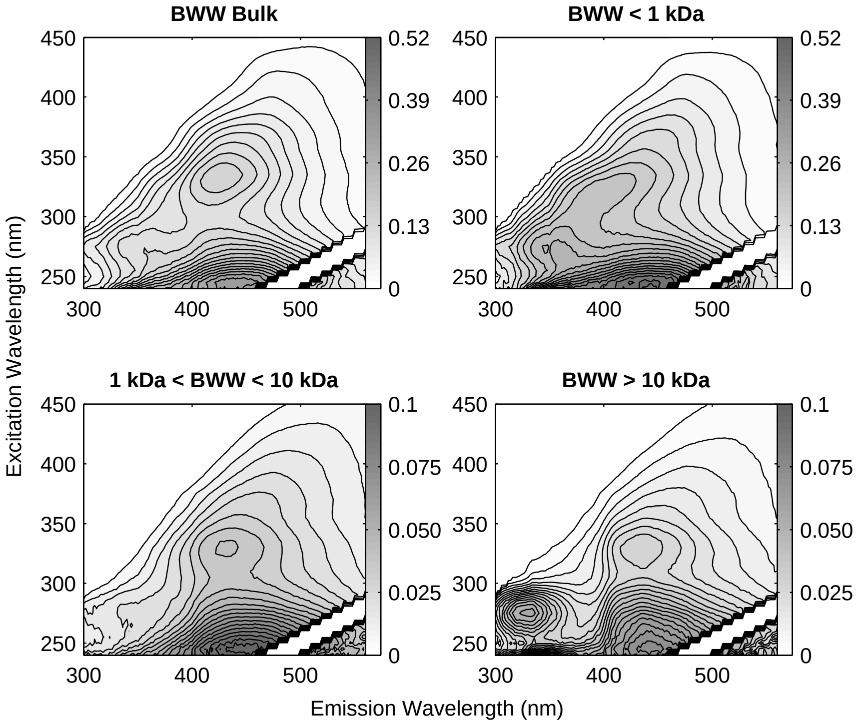


Figure 3.4 Fluorescence EEMs for BWW and each size fraction. Each EEM is the average EEM from three replicates. Note the different scales used for the various samples

The fluorescence quantum yields (Φ_f) as a function of excitation wavelength are presented in Figures 3.5a and 3.5b and show that for both OM sources the Φ_f increases with decreasing molecular size. The bulk quantum yield is influenced by the relative contribution of each size fraction. Low molecular weight species make the greatest relative contribution towards the overall fluorescence of unfractionated BWW and the Φ_f of this unfractionated sample falls in between that of the <1 kDa and 1-10 kDa fractions. Unfractionated SRNOM Φ_f, however, is closer to that of the mid-size fraction. The maximum Φ_f occurred at an excitation of 350 nm for all samples except for the <1 kDa BWW (360 nm). The position of max Φ_f with respect to excitation energy appears to be independent of size, as suggested elsewhere.[31]

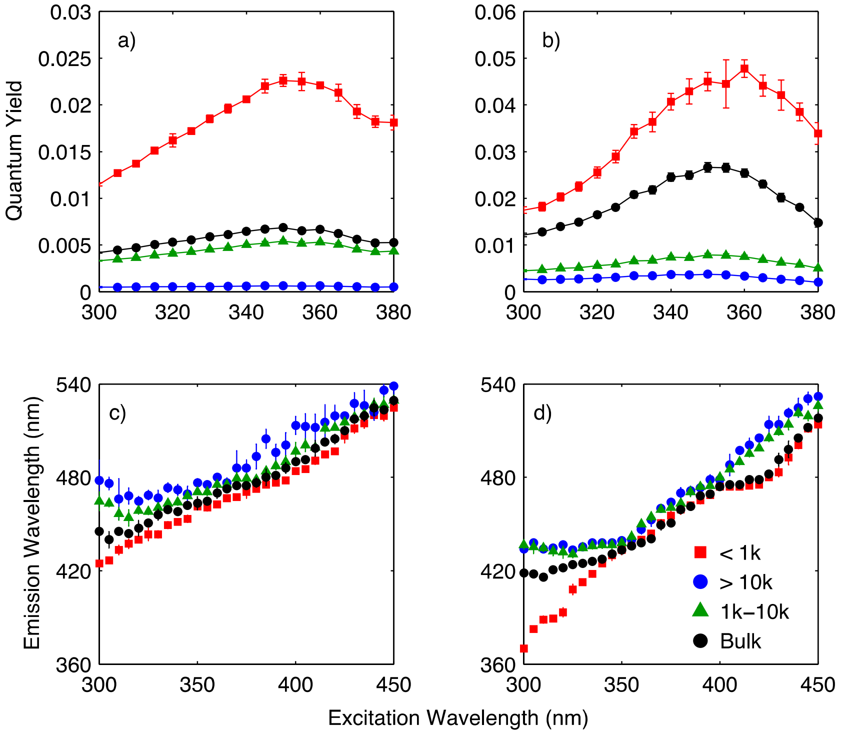


Figure 3.5 Fluorescence quantum yields as a function of excitation wavelength is presented for each size fraction in a) SRNOM and b) BWW. Peak emission wavelength as a function of excitation wavelength is depicted for each size fraction for c) SRNOM and d) BWW.

The correlation between excitation wavelength (λ_{ex}) and peak emission wavelength (λ_{em}) has been suggested as evidence for CT interactions as the source of fluorescence in the visible range[30,31] and is depicted in Figures 3.5c and 3.5d. Such interpretation is derived from the assumption that a red shift in the peak emission with increasing excitation wavelength indicates that the set of fluorophores emitting upon excitation with longer wavelengths represents a subgroup of those excited at shorter wavelengths. This behavior is in contrast to regions where λ_{em} is independent of λ_{ex} and is attributed to individual fluorophores that behave independently from other processes, i.e., emission from local excited states.[30,31] All fractions exhibit a region where excitation and emission wavelengths are correlated. The larger molecular weight fractions (>10 kDa and 1-10 kDa) both show a region where λ_{em} is independent of λ_{ex}, which occurs at λ_{ex}<350 nm. Both <1 kDa fractions show a continued correlation at λ_{ex}<350 nm. The bulk waters display characteristics of both size fraction behaviors. Past work suggests that the region where λ_{em} is independent of λ_{ex} occurs at excitation wavelengths less than that of the maximum Φ_f.[30,31] These samples suggest that this trend is violated in the low molecular weight fractions, which may be an indication of different fluorescence mechanisms.

The wastewater samples exhibit a behavior not reported elsewhere. The BWW <1 kDa fraction has a small shoulder at λ_{ex} of 310-320 nm (Figure 3.5d), which may be due to local state emissions from the blue-shifted fluorescence commonly attributed to the Peak M region.[55] A fluorescence plateau in the Peak M region is apparent in Figure 3.4. Finally, both the unfractionated and <1 kDa BWW fraction exhibit a region where emission wavelength is independent of λ_{ex} between 400 and 420 nm, suggesting the presence of low molecular weight species emitting from local states that dominate the fluorescence relative to charge transfer interactions.

<u>*Formation of reactive intermediates.*</u> Figure 3.6 shows the Φ for the reactive intermediates measured ($HO^{\bullet}$, $^1O_2$) and TMP degradation (^3OM) as well as the maximum fluorescence quantum yield. In this figure, quantum yields for each photochemical process have been normalized to the quantum yield measured in the corresponding bulk water for comparison on the same numeric scale (all Φ values are shown in Tables 3.1 and 3.2).

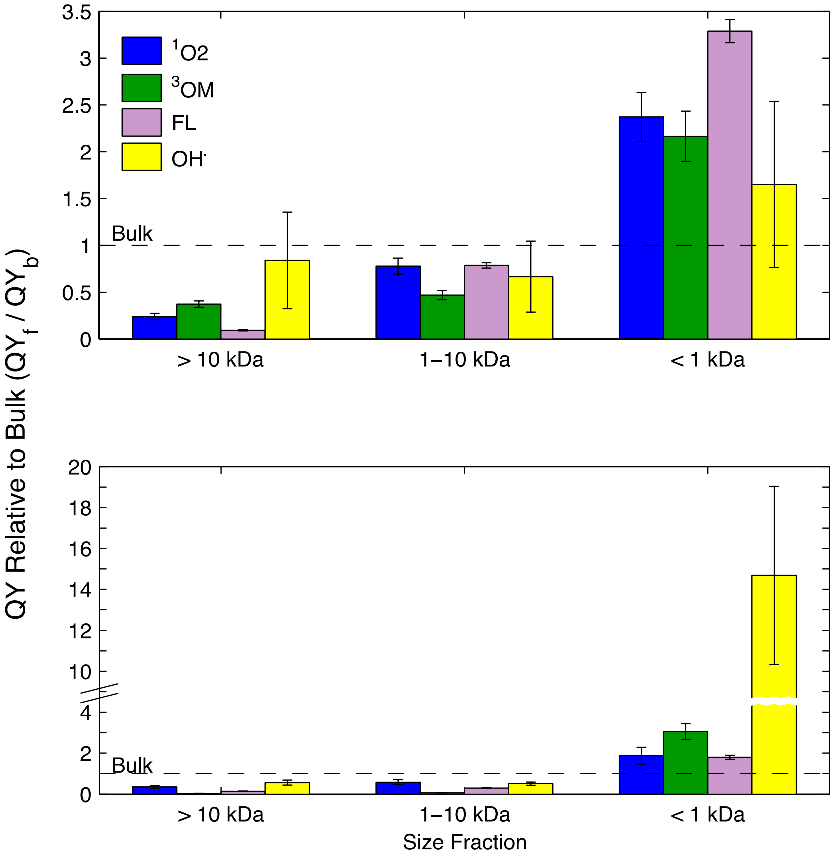


Figure 3.6 Quantum yields for the formation of reactive intermediates ($^1O_2$, $HO^{\bullet}$), TMP degradation (^3OM), and maximum fluorescence for the different size fractions normalized to the values of the unfractionated samples of SRNOM and BWW. Quantum yields for bulk samples are assigned a value of 1 for each specific species and the relative values for the fractions are displayed

Clear trends emerge for both sets of samples (SRNOM, BWW) where quantum yields increase with decreasing size. In all cases, the <1 kDa fraction shows the highest value and the >10 kDa the lowest. These trends for $HO^{\bullet}$ and $^1O_2$ have previously been reported for similar samples[28,38] and the same behavior is shown here for the ^3OM, consistent with results reported for fulvic acid[40] and humic acid triplet states.[35] These results, together with fluorescence, suggest that the lower MW fractions are more reactive in terms of fluorescence relaxation and photochemical production of RIs.

Relation between measured properties. Since the various parameters discussed above increase with decreasing size, it is not surprising that monotonic positive relations emerge when comparing the formation efficiency of the various reactive species and optical properties. Such trends are shown in Figures 3.7 and 3.8, where the efficiency of the various photochemical processes positively correlates to S and fluorescence index (FI) values, respectively (similar trends were found for E2:E3, not shown).

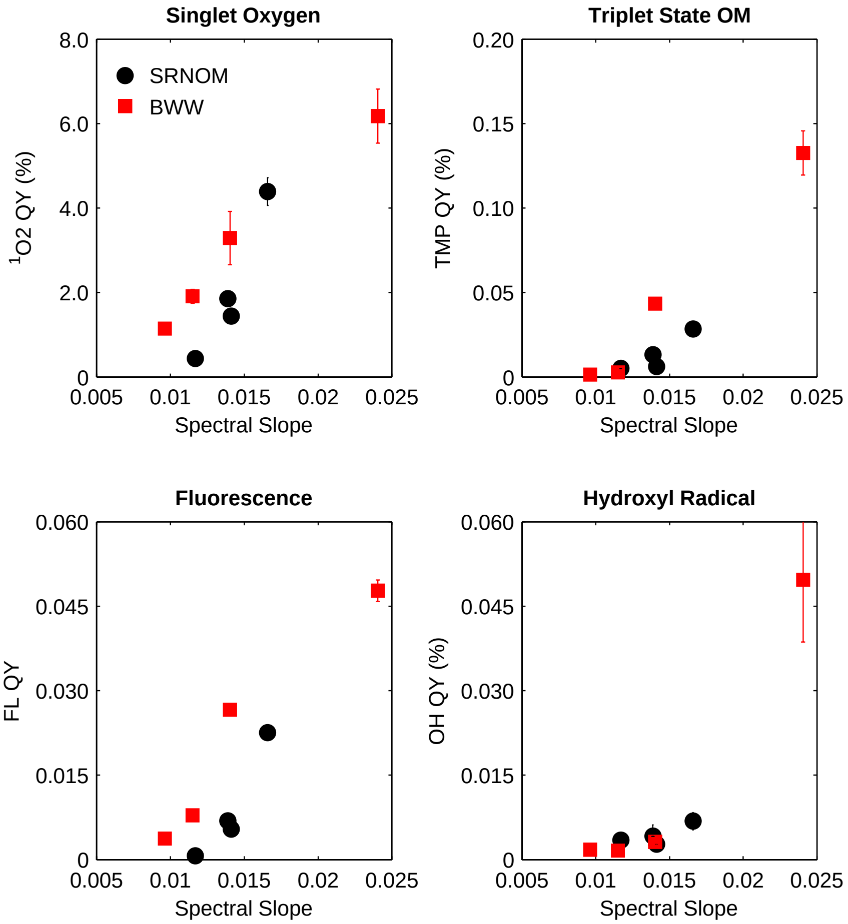


Figure 3.7 Quantum yields of the various processes measured as a function of sample spectral slope. Values for the unfractionated samples and three size fractions for both SRNOM and BWW are displayed.

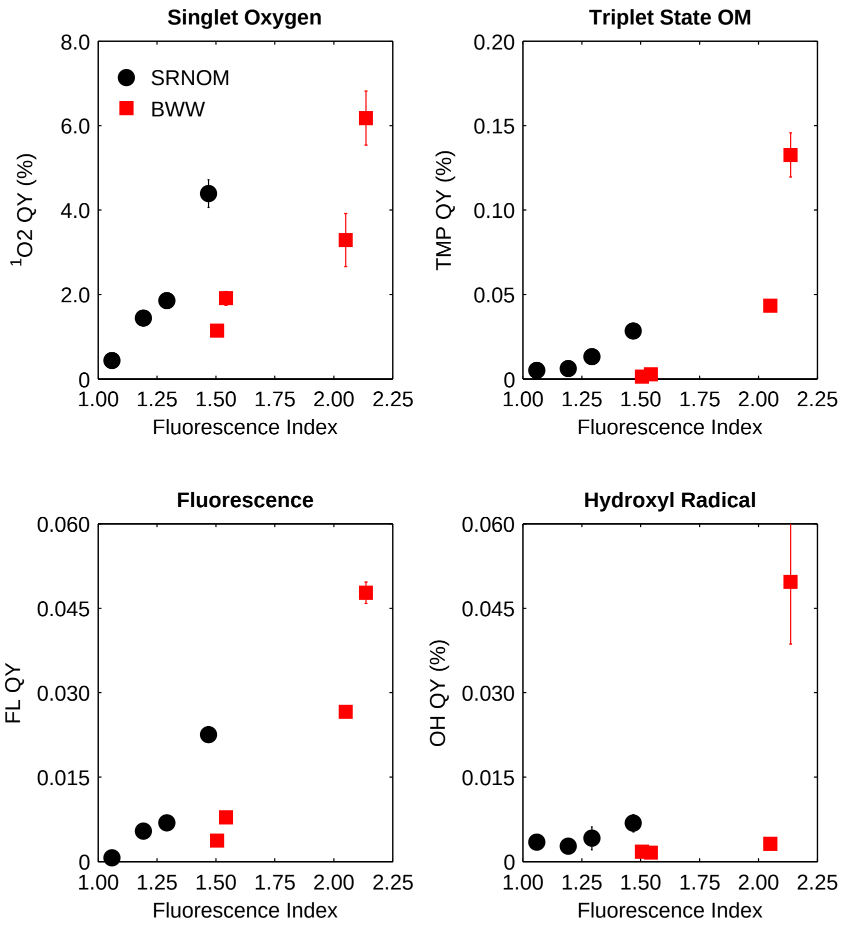


Figure 3.8 Quantum yields as a function of fluorescence index. Values for the unfractionated samples and three size fractions for both SRNOM and BWW are displayed.

While the trends observed across sample sets may vary, the emergence of such clear trends within each set of size fractions suggests that similar underlying mechanisms are involved. Furthermore, Figure 3.9 shows the efficiency of the various processes as a function of average elution time for the SEC measurements. A clear positive relationship is observed where increasing elution time (indicating lower average sizes) corresponds to higher quantum efficiencies. It should be noted that both sets of samples show very similar changes as a function of elution time, despite their distinct composition, indicating that size plays a major role in the fate of light-induced processes.

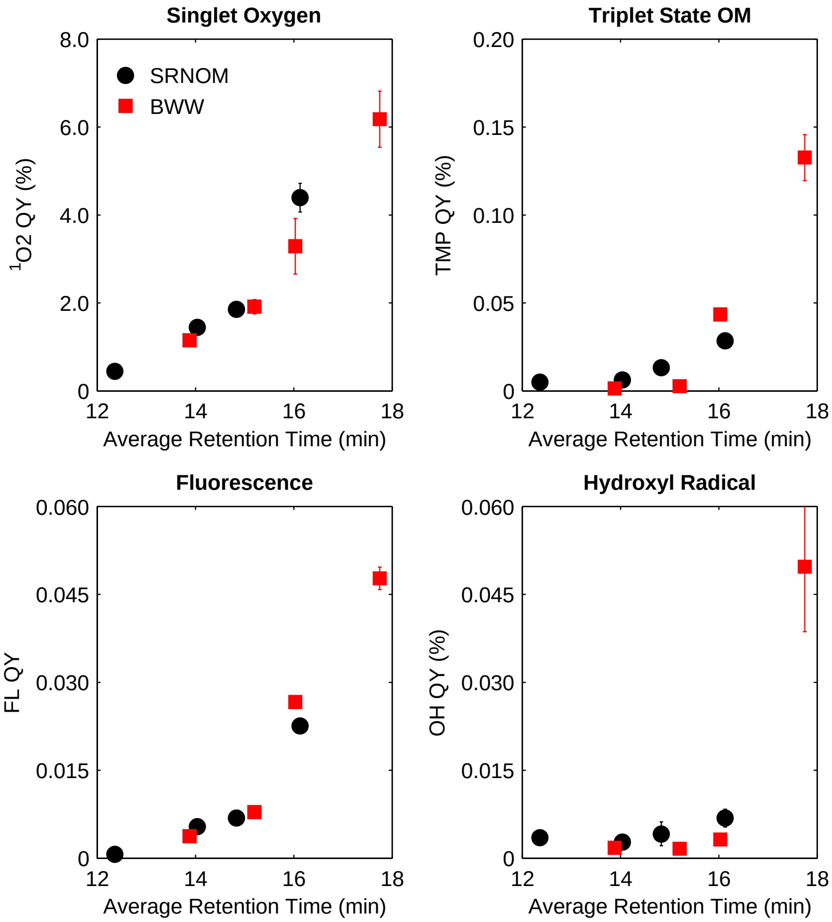


Figure 3.9 Quantum yields as a function of SEC retention time weighted average. Values for the unfractionated samples and three size fractions for both SRNOM and BWW are displayed

Interpretation of observed results. Various conceptual interpretations could satisfactorily explain the results presented here. First, differences in the photophysics of large and small size fractions could be attributed simply to differences in composition among the different fractions. In this case, it could be argued that chromophoric species displaying high absorbance but relatively low fluorescence yields are preferentially found in the larger size fractions with the opposite trend expected in the lower fractions. While this phenomenon could have an impact, it is unlikely to be the only factor at play, considering how consistent the correlations between size and photochemical activity appear to be, even when considering OM samples of very different origins.

Another possible explanation derives from the fact that DOM has exhibited correlations between molecular weight fractions and aromaticity, which may be an indication of greater degrees of conjugation in the higher molecular weight fraction.[56] Such increased conjugation would lead to the formation of singlet excited states that possess lower energies as compared to those present in smaller molecules. Such lower energy states are more prone to non-radiative decay[30] and are also expected to have shorter lifetimes.[31] Furthermore, the ^{3}OM formed are likely to have lower energies as well since they are formed via ISC from singlets of relatively low energy. Thus, they will display shorter lifetimes than those of smaller molecules, which decreases the probability of reacting with species of interest before decaying to the ground state. This would lead to the lower formation rates of $^1O_2$ and lower TMP degradation rates observed.

Another factor contributing to the observed results is that increasing size may lead to an increased number of donor-acceptor pairs, leading to enhanced CT processes (see Scheme 1). Such transfers would be reflected in longer absorbance spectrum tailing and associated decreased S values, as observed here, indicating that spectral characteristics could serve as indicators of increased prevalence of such processes. It should be noted, however, that CT processes are likely still present in lower MW fractions as suggested by the fluorescence data. Such processes may compete with both fluorescence and ISC, in accordance with the model developed by Sharpless[22,23] for explaining the observed relation between E2:E3 ratio and $^1O_2$ quantum yields. This model is further supported by a decrease in fluorescence quantum yields for larger size fractions, while the impact on the relative prevalence of ISC is reflected by a decrease in the formation of not only $^1O_2$ but also triplets (i.e. lower TMP decay rates). Similarly, $HO^\bullet$ formation decreases with increasing size due to an increase in radiationless decay and CT processes, since this leads to lower probability of excited species (whether in the singlet or triplet state) forming $HO^\bullet$ radicals before decaying to the ground state.

It is likely that a combination of these factors affect the OM photophysics to different degrees. The conceptual explanations presented allow for a new way of understanding the intramolecular photophysics of OM where fluorescence and ISC are not competing processes to the extent that one would significantly inhibit the other. Instead, they may both be similarly affected by the increase in radiationless decay (i.e. IC processes) or other processes (e.g. CT) observed to increase with increasing size and that inhibit the occurrence of measurable (and generally more environmentally relevant) photophysical and photochemical processes. While precise quantification of the phenomena discussed above remains elusive, the qualitative descriptions presented provide a satisfactory explanation to the general trends observed here and elsewhere.

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Chapter 4 Relative impact of light screening and photosensitizers on *E. faecalis* inactivation in surface waters

Abstract

Sunlight irradiation is known to contribute to microbial inactivation in natural treatment systems (NTS) and surface waters via both direct and indirect processes. Direct and endogenous photoinactivation is hindered with increasing depth due to light screening by natural and anthropogenic organic matter (OM). Additionally, indirect exogenous processes, predominantly involving singlet oxygen ($^1O_2$), are anticipated to be important for disinfection in NTS, with OM serving as a photosensitizer. This study investigated the influence of OM on endogenous and exogenous photoinactivation of *Enterococcus faecalis* upon exposure to simulated sunlight in surface waters, wastewater, and in the presence of natural organic matter (OM) isolates or a $^1O_2$ sensitizer, methylene blue. The presence of OM resulted in increases inactivation rates of 17-67% compared to sensitizer-free experiments. This suggests that the role of OM as a photosensitizer for the formation of reactive intermediates is greater than its negative impact due to light screening at levels typically found in NTS. Specifically, an increase in light absorbance (particularly at shorter wavelengths) are estimated to reduce direct and endogenous processes by up to 41% under our experimental conditions while the presence of external photosensitizers enhances exogenous inactivation processes. An approach is presented to help estimate the endogenous and exogenous inactivation rates of *E. faecalis* as a function of depth in the presence of OM based on common optical measurements. These results are important in informing the design parameters (e.g. depth, retention time) of NTS by providing a measure of inactivation kinetics as impacted by depth in the presence of diverse types of OM.

Introduction

All surface waters contain a variety of microorganisms and contaminants that vary depending on waste inputs and various environmental conditions. Both wastewater-impacted natural water bodies and systems built for treating these streams are expected to undergo a decrease in the concentration of pathogens and other contaminants over time due to various naturally-occurring processes. Treatment ponds and constructed wetlands are examples of technologies that rely on natural processes, thus called natural treatment systems (NTS), to achieve treatment levels that are comparable to secondary treatment in conventional treatment systems such as activated sludge.[1] Treatment is achieved with low or no chemical and energy inputs, deeming them particularly appropriate for low-resource or isolated communities. Pathogens are removed in these systems to levels that typically range between 0.5-3 $\log_{10}$ via various processes including sedimentation, predation, adsorption and natural die-off as well as photoinduced inactivation processes.[2–5]

Photoinduced processes include both exogenous and endogenous mechanisms, and the latter can be further classified as direct and indirect (sensitized) processes.[6] In the case of direct processes, solar irradiance is absorbed by the target compound in the microbe, causing (or contributing to) damage that leads to inactivation, as in the case of DNA damage upon UV exposure.[7] Indirect endogenous processes involve light absorption by microbial structures or chromophores that are not necessarily damaged but that act as internal photosensitizers in the formation of reactive species that lead to inactivating damage.[6] Previous research (conducted in seawater samples under oxic conditions) has pointed to oxidative stress, rather than membrane or direct DNA damage, as a dominant mechanism in endogenous damage under simulated sunlight.[8]

Light absorbance by different water constituents would generally reduce the inactivation efficiency by preventing direct interaction between the incoming light and the target microorganisms, thus creating a shielding effect. Such shielding effect would become more important with increasing distance from the light source or depth (in the case of solar irradiation). This effect is expected to be more prevalent in NTS than within engineered systems or water undergoing SODIS for drinking purposes, since higher loadings of organic matter (OM) present in NTS systems will increase light absorbance. While such light absorbance is likely to have a net negative effect upon direct and endogenous microbe inactivation, light absorbance by chromophoric OM can lead to the formation of reactive intermediates (RI),[9–12] including singlet oxygen ($^1O_2$), hydroxyl radical ($HO^\bullet$), and excited triplet states (^3OM), among others. Such RI can then come in contact with either suspended or particle-associated microbes, leading to indirect exogenous photoinactivation.

Several studies have reported enhanced inactivation of bacteria[6,13–15] and viruses[14,16–18] due to indirect exogenous photochemical processes, with $^1O_2$ playing a key role in microbial inactivation.[13,15–20] Given that lower wavelengths (i.e. below approximately 320 nm),[6] typically responsible for direct inactivation penetrate shorter depths relative to longer wavelengths, their contribution to microbe inactivation is expected to decrease with depth. On the other hand, longer wavelengths, although relatively unimportant in the direct inactivation of microbes, are expected to travel to greater depths and their relative contribution to disinfection via indirect photoinactivation is expected to increase with depth. As a result, knowledge regarding exogenous photochemical processes becomes increasingly relevant for the design of NTS.

In this work, inactivation of *Entorococcus faecalis* was studied under simulated sunlight. This microorganism was chosen due to its importance as an indicator of fecal contamination and of treatment efficacy, ease of use, and gaps of knowledge found in the literature regarding endogenous and exogenous inactivation kinetics. While both processes are known to take place, the relative contribution of each to overall inactivation has not been fully established, particularly for *E. faecalis*. This study examines the impacts of a variety of important OM types including wastewater OM, environmental surface waters OM, and OM isolates, as well as a singlet oxygen photosensitizer (methylene blue, MB), on the photoinactivation kinetics of *E. faecalis*. The contribution of endogenous and exogenous inactivation for each sample is presented along with a discussion on the implications for the design of NTS.

Methods

Sample preparation and characterization. Four natural water samples were collected to represent a range of water quality characteristics of surface waters or NTS. Two wastewater samples were collected (Boulder Wastewater Treatment Facility, Boulder, CO, USA): primary clarification effluent (BWW-1C) and final effluent (BWW-eff). Surface water samples were collected from Boulder Creek and Greeley Reservoir (both in Colorado, USA). These four natural samples were filtered upon arrival to the laboratory using a 0.7 μm glass fiber filter and stored at 4 °C until use. OM isolate samples included Suwannee River humic (SRHA) and fulvic acids (SRFA), obtained from the International Humic Substances Society (catalog numbers 2S101H and 1S101F, respectively), prepared at concentrations of 20 ppm. For the MB samples, a stock solution was diluted into PBS to concentrations of 0.3 and 1 ppm.

Dissolved organic carbon (DOC) measurements were conducted using a TOC-VCSH (Shimadzu Corp., Japan) analyzer and UV-Vis absorption was measured from 200 to 600 nm in 1 nm increments in a 1 cm pathlength quartz cuvette (Cary 100, Agilent Technologies CA). Furfuryl alcohol (FFA) was used as a probe for $^1O_2$ measurements, as previously described.[21,22]

Briefly, FFA was added to the samples at an initial concentration of 22.5 μM along with methanol (0.1 M) in order to quench $HO^{\bullet}$ which may be formed during light exposure. FFA degradation was monitored in 30-minute increments by extracting 1-mL aliquots in duplicate and using liquid chromatography (Agilent, 1200 LC) to measure concentration.

Enterococcus faecalis inactivation. Bacteria were grown overnight from a frozen culture in trypticase soy broth at 37 °C to a concentration of ~ 10^8 CFU/mL. In order to remove any traces of growth media, this broth was centrifuged (Eppendorf Centrifuge 5424) at 12000 RPM for 2 minutes and then re-suspended in phosphate buffered saline (PBS) after removing the supernatant. This step was repeated three times and 1 mL of the washed solution containing *E. faecalis* was added to 99 mL of each of the water matrices studied (including PBS), resulting in a concentration of ~ 10^6 CFU/mL at the beginning of the exposure experiments.

Samples were placed in a 150-mL stirred beaker (4.45 cm water depth) under a solar simulator OrielSol1A (Oriel Instruments, Stratford, CT) equipped with a 1000 W Xe lamp and a 1.5 air mass filter. Temperature was controlled for the duration of the experiment at 20 ± 3 °C by placing the beaker within a custom designed water bath with recirculating water from a cooling device. Total exposure times were 3-4 hours as necessary to achieve inactivation levels greater than 3 $\log_{10}$ for all samples. Samples taken every 30 minutes (1-mL aliquots) were diluted and plated using a spread plate method. *E. faecalis* procedures were similar to USEPA-approved Standard Methods #9230,[23] with incubation at 34 °C for 24 h immediately following plating on trypticase soy agar. The accuracy of plate counts was warranted by duplicate plating for each time step sample as well as the plating of numerous dilutions for each sample. Each experiment was repeated 2-3 times and results shown represent the average of those runs.

<u>Inactivation data analysis</u>. Experiments conducted in PBS represent sensitizer-free conditions; thus the inactivation rate observed is considered to involve direct and/or endogenous processes alone.[24] It is assumed that endogenous inactivation in PBS remains constant regardless of depth, which would be the case for a completely non-absorbing solution. In the presence of light-absorbing matter, average irradiation will decrease with depth as established by Morowitz[25] based on the Beer-Lambert law. While the original calculation deals with a single wavelength, the screening factor (SF) used here is calculated as the fraction of the incoming light that is present through the water column in the wavelength range of 290-600nm, as such

$$SF = \frac{\sum_{\lambda=290nm}^{600nm} I_L(\lambda)}{\sum_{\lambda=290nm}^{600nm} I_0(\lambda)} = \frac{\sum_{\lambda=290nm}^{600nm} I_0\left(\frac{1-10^{(-\alpha L)}}{(2.303)(\alpha L)}\right)(\lambda)}{\sum_{\lambda=290nm}^{600nm} I_0(\lambda)} \qquad (1)$$

where I_0 and I_L are the incoming radiation and the average radiation in a cell of depth L, respectively, α is the decadic absorption coefficient, and (λ) indicates the calculation is over a range of wavelengths.

In addition, an action spectrum is used to account for the fact that photoinactivation efficiency varies with wavelength. Considering both the decrease in average irradiance for different wavelengths and the action spectrum for *E. faecalis*, a weighted screening factor (WSF) is calculated as

$$WSF = \frac{\sum_{\lambda=290nm}^{600nm} PI_L(\lambda)}{\sum_{\lambda=290nm}^{600nm} PI_0(\lambda)} \qquad (2)$$

where P is the wavelength-dependent sensitivity coefficient (photoaction spectrum, $m^2/W*h$) for *E. faecalis* in DI water, which decreases with increasing wavelengths ($P = 60*e^{-0.02*\lambda} + 0.025$).[26] Thus, the WSF represents the fraction of direct and endogenous inactivation expected to take place at a given depth compared to that at the surface or within a clear medium.

On the other hand, as RI formation decreases with depth (due to light screening), exogenous processes are expected to decrease to the same degree. The wavelength dependence of exogenous inactivation is more difficult to account for because $^1O_2$ quantum yields appear to be only weakly dependent on wavelength (or at least difficult to generalize across diverse OM types)[27–31] and because other species may also play a role in inactivation. Therefore, it is assumed for the sake of this analysis that all wavelengths contribute equally to exogenous inactivation. Following this assumption, the rate of exogenous inactivation decreases to the same extent as the average light irradiance (described by SF) due to screening of photosensitizing irradiance with depth.

Results and discussion

E. faecalis inactivation rate. The results of the inactivation experiments in each water sample are presented in Fig. 4.1 as the $\log_{10}$ inactivation of *Enterococcus faecalis* as a function of irradiation time under simulated sunlight. These results can also be reported as inactivation as a function of applied dose or fluence (in MJ m^{-2}) over the range 290-600 nm based on the irradiance of the solar simulator (Fig B.1). All samples studied exhibit a lag before any inactivation is observed. To account for this behavior, the data were fitted using the equation[32]

$$\frac{N}{N_0} = 1 - (1 - e^{-kt})^m \qquad (3)$$

where N_0 and N represent respectively the concentration of culturable bacteria in CFU/mL at the beginning of the experiment and at time t, k represents the first order inactivation rate, and m is a fitting parameter. The k values calculated from these fits are reported in Table 4.1 and the error reported represents the 95% confidence interval for these fitted k values. The lag time was calculated as[32]

$$t_{lag} = \frac{\ln m}{k} \quad (4)$$

The inactivation rate observed for the experiments conducted in PBS are assumed to be representative of endogenous processes, i.e., not involving exogenous photosensitizers. The inactivation rate constant measured in these experiments (4.81 h^{-1}) was higher than that reported for E. faecalis in PBS by Kadir et al. (2.15 h^{-1}).[15] This difference is likely due in part to their use of an atmospheric attenuation filter, which blocks the shorter wavelengths in the solar simulator irradiance spectrum to a greater degree than the air mass filter alone used here. Since the distinction between direct and indirect endogenous processes is not possible from the data obtained here and the environmental implications of such knowledge are limited (i.e. conditions affecting these processes can be difficult to assess or modify in NTS), the subsequent discussion is focused on the distinction between bulk endogenous and exogenous processes.

In the presence of OM, the inactivation rates are 17-67 % higher and the lag times up to 54 % lower than those observed in PBS (Table 4.1). These results can be explained by the fact that OM serves as an exogenous photosensitizer in the formation of RI. Although OM can hinder endogenous processes by shielding microbes from irradiation, particularly in the short-wavelength range most critical for inactivation,[6] the role of OM as a photosensitizer increases inactivation to a greater degree that it hinders it.

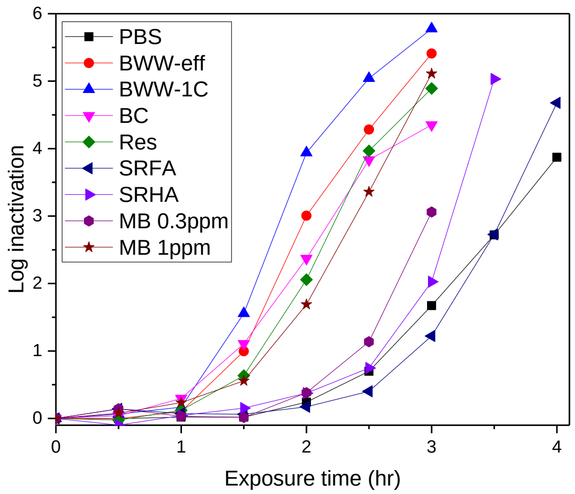

Figure 4.1. ***E. faecalis*** **$\log_{10}$ inactivation as a function of simulated solar irradiation time.**

Interestingly samples containing OM isolates exhibited less enhanced inactivation compared to the wastewater and surface waters samples, or methylene blue. This behavior may be due to relatively high absorbance by OM isolates (lower WSF) compared to their RI formation rates (e.g. $[^1O_2]_{SS}$ in Table 4.1). For example, the irradiation time required to reach 3-$\log_{10}$ inactivation ($t_{3\text{-log}}$), shows a negative correlation to the ratio of $[^1O_2]_{SS}$/ABS300 (i.e. the ratio of the bulk steady-state $^1O_2$ concentration over the absorbance at 300 nm), as shown in Fig B.2. Since increasing RI will tend to enhance inactivation while light screening would generally decrease it, it makes sense that the irradiation time or dose required for inactivation would decrease (due to higher inactivation rates) as this ratio increases.

Table 4.1. Inactivation kinetics and water quality parameters for the studied samples

Label	Sample description	Inactivation rate constant, k (h^{-1})	Lag time (h)	DOC (mg/L)	Weighted screening factor (WSF)	$[^1O_2]_{SS,\ ave}$ × 10^{13} (M)
PBS	Phosphate buffered saline	4.81 (0.44)	2.18	0.0	1.00*	n.a.
BWW-eff	Boulder WWTF effluent	6.68 (1.13)	1.07	6.9	0.92	1.66
BWW-1C	Boulder WWTF primary clarifier	8.05 (1.79)	1.00	8.5	0.90	2.07
BC	Boulder Creek	5.94 (0.84)	1.05	4.7	0.90	1.98
Res	Greeley Reservoir	7.86 (0.96)	1.36	6.7	0.96	0.88
SRFA	Suwannee River fulvic acid (20 ppm)	5.61 (0.44)	2.28	8.6	0.78	2.62
SRHA	Suwannee River humic acid (20 ppm)	6.48 (1.26)	2.04	8.2	0.59	3.11
MB 0.3ppm	Methylene blue (0.3 ppm)	6.74 (3.32)	2.01	0.0	1.00*	1.04
MB 1ppm	Methylene blue (1 ppm)	7.28 (0.91)	1.43	0.1	1.00*	4.77

* Assumed due to negligible light absorbance over wavelength range of 290-600 nm

Relative contribution of exogenous inactivation. Knowing the overall inactivation rate constants (k, from Equation 1) and calculating the contribution of endogenous processes under our experimental conditions (k_{end}), the balance of the inactivation can be assumed to be the result of exogenous processes (k_{exo}),[24] calculated as such

$$k = k_{end} + k_{exo} = k_{PBS}(WSF) + k_{exo} \quad (5)$$

The results of this analysis are shown in Fig. 4.2, which presents the overall inactivation rate constant of each sample as well as the contribution of both endogenous and exogenous mechanisms, with the assumption that inactivation taking place in PBS (k_{PBS}) is due to endogenous processes alone. All the other samples display a decrease in the endogenous contribution as compared to PBS due to light screening. At the same time, the contribution of exogenous processes is shown to be greater than the corresponding decrease in endogenous activity for each sample, thus leading to overall inactivation rates that are higher than those in non-RI producing samples.

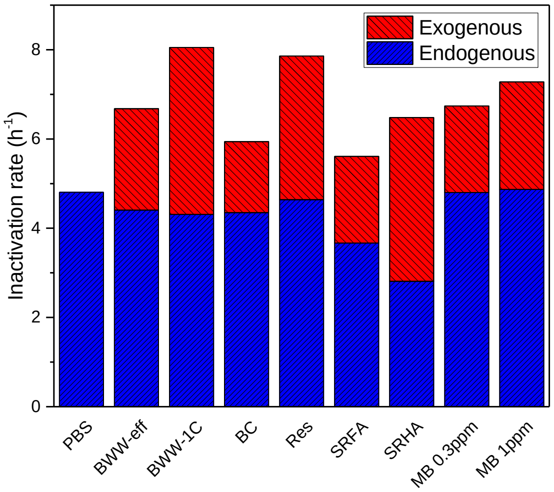

Figure 4.2. Inactivation rate constants detailing contribution from endogenous and exogenous pathways. Inactivation in PBS is assumed to represent endogenous processes alone which decrease for other samples due to light screening.

Interestingly, the rate of exogenous inactivation does not seem to correlate strongly with $[^1O_2]_{SS}$ (Fig B.3), despite the fact that previous reports had pointed to this molecule as being very important in the inactivation of microbes.[13,15–20] While these results do not rule out the role of $^1O_2$ as an important species in microbial inactivation, they indicate that $[^1O_2]_{SS}$ may be a poor predictor of inactivation kinetics. Reasons for such lack of correlation may point to the complexity of exogenous inactivation processes for *E. faecalis*. Interactions between the photosensitizer and the target microbe, including proximity and membrane permeability, may play a role. For example, previous reports have shown that increased particle association between OM and viruses increased inactivation kinetics.[33] Membrane permeability could also play a role, where smaller sensitizers (that could potentially further penetrate the membrane) would enhance inactivation. Both of these phenomena, particle association and membrane permeability, are also affected by surface charges which further complicates the interactions between RI and the target microorganisms.

Different degrees of quenching/scavenging of RI by different types of OM may also impact the concentration of $^1O_2$ that the microbes actually experience and such variability may not be fully captured by the $^1O_2$ probe used here (i.e. FFA).[34] Other RI, e.g., $HO^•$ and OM excited triplet states could potentially play a role,[35] yet their behavior would be similarly affected by the complexities mentioned above. The role of $HO^•$ was specifically assessed here by measuring inactivation in the presence of environmentally relevant concentrations (1 mM) of NO_3 (a selective $HO^•$ sensitizer) and no difference was observed compared to inactivation rates in PBS (data not shown). Under these conditions, $HO^•$ is expected to be in the order of 10^{-14} M,[36] which is 1-2 orders of magnitude higher than it would be in the environment due to the presence of scavengers such as OM.[22,37] This points to the fact that $HO^•$ is unlikely to play a significant role in *E. faecalis* inactivation under environmentally relevant conditions, at least compared to other processes within the timeframes tested. Synergistic effects between the various processes (i.e. direct and indirect endogenous and exogenous) may also play a role[38] but are yet to be fully elucidated. As a result of the complexity of these interactions, attempts to estimate inactivation rates using second order reaction rate constants between $^1O_2$ and *E. faecalis* or CT values (assuming Chick's law behavior) is likely to yield erroneous estimations.

However, one important result derived from this analysis is that the most environmentally relevant samples (i.e. surface and wastewaters) display a relatively narrow range of inactivation rates and $t_{3\text{-log}}$ values. Samples derived from surface and wastewaters all require solar exposure dose of 3-4 MJ m^{-2} to reach 3-log inactivation under the conditions tested. While this observation needs to be tested in full-scale systems, it provides some confidence that the hydraulic retention times necessary to reach the desired treatment level in NTS, will not change dramatically as a function of influent water characteristics.

Inactivation as a function of depth. Both the light absorption spectrum of OM and the action spectrum of *E. faecalis* show a decrease with increasing wavelength, which results in the most effective inactivating wavelengths being preferentially blocked. As a result, the WSF decreases more sharply as a function of depth than the overall SF (e.g. Fig. B.4). As described in the Methodology section, endogenous processes are assumed to decrease with depth as described by the WSF while the exogenous is assumed to decrease with the SF, while the the fitting value m (as calculated in Eq. 1) is assumed to remain constant irrespective of depth for a given sample. This assumption is justified on the basis that m is a factor used to fit the inactivation curves (in Fig. 4.1) assuming a multi-target model,[32] and the factors it describes (e.g. number of targets) are not expected to be vary with depth. Following this assumption and based on the dose-response kinetics of endogenous and exogenous inactivation processes (k_{end} and k_{exo}), rates at different depths can be estimated.

Fig 4.3 illustrates the results of two samples, displaying the lowest (BC) and highest (BWW-1C) inactivation rates among the natural water samples studied here. The data in this figure model the decrease in the overall inactivation rate as a function of depth for these two samples, as well as the relative contributions of endogenous and exogenous processes. For both samples, rates decrease to half of the rate at the surface, at a depth of 40-50 cm. As expected, the endogenous processes decrease more sharply with depth, leading to an increase in the relative contribution of exogenous processes, even as the overall rate decreases. In the case of BWW-1C, exogenous processes may be responsible for 46-55 % of the total inactivation but may account for only 26-35 % of inactivation in the BC sample within one meter of the water surface.

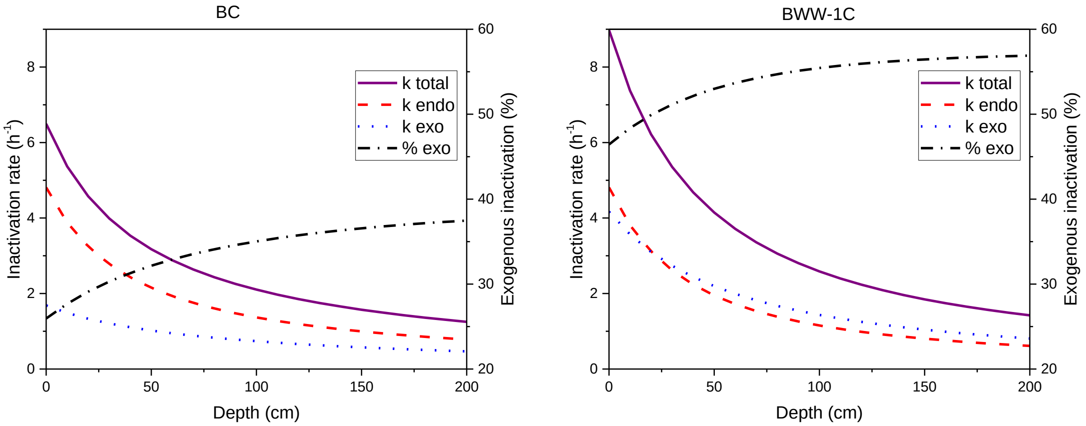

Figure 4.3. Calculated overall inactivation rate and relative contribution of endogenous and exogenous processes as a function of depth for Boulder Creek (BC) and primary clarifier effluent (BWW-1C) samples. Contribution of exogenous processes to overall inactivation plotted on secondary axis.

Environmental implications

The data presented provide an estimation of inactivation kinetics for *E. faecalis* as well as an approach for estimating the contribution of different mechanisms, i.e. endogenous and exogenous, which can be applied to other microorganisms of interest. These bench-scale experiments provide useful information that can be complemented by field studies to gain a wider understanding of the factors affecting microbial inactivation. For example, while the times required to reach a certain degree of inactivation in the natural samples fall within a relatively narrow range (i.e. 1.9-2.2 hours to achieve 3-$\log_{10}$ removal under our experimental conditions), this may not be the case in full-scale systems due to variability in flow/mixing or seasonal/diurnal fluctuations in environmental conditions that may affect inactivation kinetics. Furthermore, indigenous bacteria are more resistant to sunlight disinfection than pure strains grown in the laboratory. Thus, further research that utilizes indigenous and pathogenic microorganisms (as opposed to lab-cultured indicators) would improve our estimations of disinfection kinetics under environmental conditions.

During NTS design, the microorganisms and conditions that require the greatest exposure times to achieve a given goal of treatment would dictate the required HRT. However, it is important to acknowledge that the necessary exposure times will increase with increasing depth due to decreasing photoinactivation rates. This points to the notion that HRT should not be increased by increasing depth alone, but that a maximum or optimal depth should be established based on optimizing the mechanisms of solar inactivation given the absorbance conditions in the NTS.[24] Furthermore, the NTS design geometry must take into account not only the desired treatment level (influenced by HRT and depth) but also land availability/cost.[39] While this is an optimization question that is beyond the scope of this article, improved knowledge of inactivation kinetics and mechanisms of various microbes of concern would serve as more reliable input variables for the calculation of design parameters.

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Supporting Information

This information is shown in Appendix B: Supporting Information.

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Chapter 5 Challenges and opportunities for wastewater treatment and reuse in developing communities

Introduction

Worldwide population growth and increased per capita consumption of natural resources have led to scarcity of valuable resources as well as the associated increases in waste streams.[1] The impacts of such changes in the global flow of materials are particularly noticeable in the developing world due to a combination of demographic, socio-economic, and environmental factors. For instance, population growth is taking place at a greater rate in developing countries around the world, whereas populations have plateaued or even slightly decreased in the developed world. Furthermore, in many countries, the development of infrastructure to accommodate for the changing needs of the population and regulations to control the extraction and disposal of materials have not been adequate. This has led to the overexploitation of natural resources and uncontrolled waste disposal, which have detrimental impacts upon environmental and health conditions.[1]

As a necessary (yet often scarce) resource, water is of particular concern and presents an important example of the problems described above. Water is fundamental to sustain life as it is necessary not only for hydration but also for cooking, hygiene practices, and irrigation. Particularly in rural communities, agricultural irrigation accounts for a majority of water necessities. On the other hand, water can also become a vector for diseases caused by pathogenic or toxic agents. This is particularly true of waters receiving untreated (or improperly treated) waste streams. In the case of wastewater streams, their magnitude can be assumed to increase in relation to increases in water consumption, particularly in sewered systems. Thus, whereas increased water usage has generated shortages in supply, improper management of waste has further compromised the quality of freshwater sources.

Due to the high microbial concentrations present in raw sewage, the removal of pathogens is one of the primary objectives in wastewater treatment (WWT). Without it, soil, crops, and water sources (both groundwater and surface water bodies) may become contaminated when coming in contact with untreated water streams leading to detrimental health and environmental impacts. Thus, it becomes clear that the development, optimization, and introduction of decentralized, non-conventional wastewater treatment systems that do not rely on high energy, chemical, and labor inputs and that are socially and environmentally sound would be highly beneficial to those communities that do not have access to conventional treatment technologies common in more developed regions. Waste stabilization ponds (WSP) and constructed wetlands (CW) are some of the systems considered to be appropriate for this purpose, evidenced in part by the fact that they are already widely used for treating waste streams in wide range of conditions and geographical locations.[2]

Furthermore, the reuse or recycling of materials presents an opportunity to turn waste into valuable resources, thus addressing both the issue of scarcity and that of increased waste. The use of wastewater for irrigation purposes is one of such instances which allows the reuse of both water and nutrients, potentially minimizing environmental and health impacts of wastewater and improving (or decreasing the cost of) agricultural practices. This concept is certainly not new and has been practiced in both the developing and developed world under different circumstances. For instance, in the United States and Europe, strict regulations have been implemented to ensure treated wastewater being reused meets health and environmental standards, while exploiting the benefits of this practice. However, such practices in the developing regions mostly take place in an informal (possibly illegal) manner, and thus remain unregulated. Due to lack of regulation and its predominantly informal nature, quantification of the extent of this practice remains a difficult task, however it is estimated that approximately 20 million hectares worldwide are irrigated using raw, or partially diluted, sewage.[3] Issues surrounding this topic in developing countries are discussed in this chapter by considering both the benefits and potential health impacts of such practices, realistic goals for minimizing such impacts, and various options for improving wastewater management, including treatment in natural treatment systems (NTS).

Benefits and risks of wastewater irrigation

When assessing the issue of wastewater irrigated crops, it is important to consider both the potential benefits and risks associated with this practice. The benefits of using wastewater as a source for irrigation are directly linked to the necessity of different communities to meet their basic needs. A water supply that is adequate in terms of both quantity and reliability is a valuable resource for communities which depend on agriculture, either for subsistence or commercial purposes. Such a fundamental resource may not always be available, possibly due to environmental reasons, e.g., in arid or semi-arid areas, or more complex issues regarding, for instance, increased water extraction/allocation for urban centers. The latter case presents an example where a steady supply of water to urban regions effectively guarantees a reliable supply of wastewater for urban, peri-urban, or nearby rural agriculture. This presents farmers an opportunity to address their water needs in a reliable and relatively inexpensive way. Sufficient water available for irrigation enables the adequate supply of crops, potentially enhancing food security and nutrition for farmers and communities.[4] Furthermore, the increased organic content in the soil resulting from wastewater application enhances its water-holding capacity, thus potentially requiring less water than otherwise needed.[5]

Urban agriculture, which may significantly depend on wastewater for irrigation, is becoming an important economic activity and an important contribution to vegetable supply in urban centers. Economic analyses regarding the different agricultural methods have been conducted, for instance, in different regions of Africa, based on surveys and data related to revenue generated. Studies have found that year-round irrigated urban vegetable farming could move households in Dakar, Senegal[5] and Kumasi, Ghana[6] above the poverty line of $1 per day, while rainfed and/or dry-season irrigation schemes would not be sufficient to surpass this threshold, according to the latter study. Another important aspect regarding urban agriculture is the significant role it plays in supplying perishable vegetable goods to city dwellers. For instance, it is estimated that 60% of the vegetables consumed in Dakar are produced in or near the city.[7] This proximity between the point of production and consumption is of particular importance in regions that do not posses systems of sufficient capacity for refrigerated transportation and storage of vegetables. In this regard, urban agriculture can potentially present benefits not only for the farmers and their families but for urban populations as a whole by contributing to meet their food demands.

In addition, wastewater presents a significant advantage compared to freshwater due to its nutrient content, including nitrogen, phosphorus, and potassium (NPK). The economic benefits of such nutrient availability can be understood in terms of either increased yields or the value of fertilizer saved.[8] These benefits translate into increased household income for farmers and a greater food supply to the market (with the potential decrease in price related to this increased supply). In addition to the economic benefits of decreased fertilizer needs, the environmental aspects should also be considered. Production of inorganic fertilizer is an energy intensive and environmentally detrimental process due to the need for atmospheric nitrogen fixation as well as phosphorus and potassium extraction (predominantly from mining). Thus, reusing these valuable nutrients by means of wastewater irrigation contributes to decreasing the needs for inorganic fertilizer production.

Despite all its potential benefits, uncontrolled use of raw sewage for crop irrigation presents a series of health risks for farmers, consumers, and communities at large. Untreated domestic wastewater carries with it significant amounts of microorganisms, a portion of which are likely to be pathogenic agents, thus becoming a potential vector for transmittance of disease when used for irrigation. However, due to the predominantly informal nature of wastewater irrigation practices, the characteristics of the water being used can be hard to establish as would be its potential health impacts.

Studies have provided evidence of increased incidence of infection and disease in farm workers and their families as a result of exposure to raw or diluted sewage. For instance, in Ouakam in the Dakar region, Senegal, where only wastewater is available for irrigation, 60% of farmers were infected with intestinal parasites; while 40% were infected in the nearby city of Pikine, where diluted wastewater is used as a source.[5] Hookworm infections affecting as much as 80% of farm workers using wastewater were reported in Haroonabad, Pakistan; compared to infections levels of ~ 40% for farmers using other water sources. In addition, farmers who irrigate their crops with wastewater, and their children, were 2-3 times as likely to suffer from diarrheal diseases as compared to the control group.[9] In general, parasitic infections are more common among farmers who use wastewater for irrigation (and their children), especially those who do not wear shoes. Increased incidence of diarrheal diseases and bacterial infections have been observed, particularly in children, while viral and *Giardia* infections have been reported in adults coming in contact with raw or partially treated water during irrigation.[10]

Another group of concern with regards to wastewater irrigation is consumers. This group represents a greater population than farmers and their families and is estimated to be more than 10% of the world population.[11] They may not come in direct contact with raw sewage but may be directly affected (possibly without their knowledge) by the practice of wastewater irrigation. In some instances, the health risks become evident when disease outbreaks that can be traced back to wastewater usage in agriculture are reported. Such was the case of the 1987 typhoid epidemic which made hundreds of people in Dakar seriously ill.[5] In most cases, however, the direct health impacts are not as obvious and may require extensive studies to establish a correlation between the source of irrigation water and epidemiological trends in a population. However, studies point to elevated risks of helminth infections and diarrheal diseases for both children and adults while outbreaks of cholera and shigellosis (a possible cause of dysentery) have also been reported in relation to wastewater irrigation.[10] In addition, evidence of crop surfaces contaminated with protozoa has been reported, although no direct correlation to increased infection rates was established.[10] It is important to consider that consumers of raw vegetables are particularly at risk since the process of cooking may significantly decrease the potentially negative health impacts. For instance, vegetables such as lettuce, onions, and tomatoes may be eaten in salads and are usually consumed uncooked. Increased frequency of consumption of such crops has been shown to cause a twofold or higher increase in diarrheal and viral infections when irrigated with wastewater or contaminated river water.[12,13]

Suggested practices for wastewater irrigation

The question is then, while understanding the potential need and benefit of wastewater irrigation, how can health risks be reduced? While treating wastewater could be ideal if appropriate systems are available (further discussed below), other practices can be implemented to decrease health risks in the absence WWT.

Changes in the way crops are irrigated may decrease the incidence of disease for both farmers and consumers. Sprinkler and furrow or flood irrigation may have significant impacts upon the health of farmers, their families, and nearby communities as they increase their probability of being in direct contact with the wastewater. On the other hand, bubbler, drip, or trickle irrigation present a reduced risk of exposure to those surrounding the cultivated land. In addition, the use of shoes and other protective equipment by farmers and their families should be encouraged as they create barriers that minimize direct skin contact to the wastewater. Another practice that would lower the risk for consumers would be to stop irrigation a few days or weeks before harvesting. Although this method may not always be applicable, e.g. lettuce should be irrigated until the time of harvest to remain fresh, it may be useful for certain crops as it allows for pathogen die-off before consumption or handling.[14]

Crop restriction refers to the selection of crops that may include non-food crops or those that are processed or cooked before consumption. In places where agricultural practices are not well regulated it would be hard to promote such practices if the market conditions were unfavorable. However, there are several examples where shifts to farming of restricted crops have being carried out without affecting farmers in cases where the demand for these crops exists.[10] As mentioned above, cooking eradicates practically all pathogens present in vegetables due to high temperatures but other food preparation methods can significantly reduce the presence of pathogens as well. For instance, washing with water and/or disinfectant solution as well as peeling fruits and vegetables can achieve 1-2 log reduction of pathogens, while similar reductions in the amounts of helminth eggs can be achieved by use of a detergent solution and rinsing with water.[10] Such practices may be effective in reducing pathogenic infections and relatively inexpensive but rely on education of food handlers, vendors, and consumers to be effectively implemented.

It can be observed that several factors and practices may have an impact upon the health risks associated with wastewater irrigation. This demonstrates that it would be very useful to develop a comprehensive approach to minimize the prevalence of disease. Such an approach should involve all the participants in the process of food production and consumption. With sufficient information, and when possible, various practices can have a cumulative effect that result in sufficient pathogen avoidance or reduction (e.g., log 6 removal of rotavirus). Starting with the selection of crops to the way they are grown, handled, and finally consumed, the opportunities to decrease health risks should be exploited by everyone involved. Making the public aware of these issues is important, not only because knowledge is necessary for proper hygiene measures to be practiced, but also because consumers can put pressure on vegetable and cooked food vendors to practice hygienic measures themselves and ensure their food comes from a sufficiently safe source. Farmers would then have an additional incentive to practice safer irrigation methods and minimize the negative health impacts of consumers.

Wastewater treatment in natural treatment systems

Due to the high microbial concentrations present in raw sewage, the removal of pathogens is one of the primary objectives in WWT. The concentrations of microbes in WW streams may vary depending on geographic location and other conditions; however typical concentrations for thermotolerant coliforms are 10^6-10^{10}/100 mL, 10-10^3/100 mL for helminth eggs, 1-10^5 for protozoa, and 10^2-10^6 for various types of viruses.[10] NTS are considered appropriate technologies for a wide range of conditions as they are more economical to build and maintain as compared to more advanced WWT processes, while holding the potential benefits of water reuse. For example, recognizing the natural processes that take place within wetlands, they have been used for centuries as receiving bodies for wastewater.[15] However, this led in many instances to their eventual degradation when the loads applied exceeded their capacity to absorb them. Thus, attempts have been made to replicate such natural processes in a controlled setting while avoiding the potential impacts upon such valuable natural systems through the construction of artificial wetlands and ponds dedicated to perform various forms of water treatment.

The first experiments using plants as part of a water treatment process are credited to Dr. Käthe Seidel in 1952. Numerous experiments were carried out in the following years, and the first constructed wetland was built in the Netherlands in 1967, while their use spread at a relatively slow pace through the 1970s in Europe and North America.[16] In the following decades, the research, implementation, and applications of constructed wetlands would greatly expand to the point where today there are "thousands of applications in all parts of the world".[16] Constructed wetlands are generally classified into two types based on where the water surface is found with respect to the supporting media. Free water surface (FWS) wetlands, also known as surface flow wetlands, are those in which the water remains exposed while flowing above the supporting media. On the other hand, sub-surface flow (SSF) wetlands, also known as reed beds and planted soil filters, are designed in such a way that the media, e.g. sand, gravel, extends above the water surface. This latter type presents advantages with respect to FWS since it avoids possible problems associated with vector breeding and odors in open waters as well as increased surface area in the media which enhances biological growth and filtration mechanisms. On the other hand, they may be more susceptible to clogging and are not exposed to direct sunlight, which may enhance microbial inactivation in FWS wetlands. SSF CW can be further classified with respect to flow direction as vertical (upflow or downflow) or horizontal flow.

CWs have proved to be an appropriate technology for the treatment of wastewater streams derived from various sources, including municipal and domestic wastewater (these being the most common), various industrial applications (e.g. chemical, paper, and textile factories), runoff streams (e.g. airports, highways, greenhouses), agricultural wastewater, as well as landfill leachate.[17] Raw sewage contains high levels of suspended solids (120-400 mg/L), organic matter that would impose an oxygen demand upon receiving bodies (110-350 mg/L BOD_5), nutrients (20-70 mg/L-N, 4-12 mg/L-P), numerous pathogens (bacteria, viruses, protozoa, and helminths), as well as other contaminants of concern.[18] However, in most instances some form of pre-treatment (e.g. septic tank, sedimentation pond) is required before entering the CW, leading to influent values lower than those present in raw WW. The mechanisms leading to the removal of suspended solids in CWs include sedimentation and filtration with average removal efficiencies of approximately 70%. BOD and nutrient removal take place due to a combination of physical and biological processes. Microbial degradation and sedimentation are the dominant processes in BOD removal, which leads to an average efficiency of ~80% for wastewater influents containing more than 40 mg/L BOD_5. Nitrogen removal (average TN removal ~40%) takes place due to ammonification followed by microbial nitrification/denitrification or by ammonia volatilization, while plant uptake usually plays a smaller role. Due to a lack of biological processes leading to phosphorus volatilization, reduction in total phosphorus in the effluent (~40%) occurs due to soil sorption (possibly aided by adsorption-precipitation reactions with other water constituents) or by plant uptake. In the case of pathogens, mechanical and biological processes lead to reductions in pathogen concentrations ranging between 0.5 to 3 log,[10,17,19] although they are rarel designed with this specific purpose in mind.[20] Higher removals of coliforms are usually found in hybrid systems combining some form of FWS and SSF CWs. The removal efficiency has been found to be related to various factors, including hydraulic retention time (HRT), presence of vegetation, porous media, and temperature, among others.[20] The physical and chemical mechanisms responsible for microbe removal include filtration, sedimentation, oxidation, UV radiation (for FWS CWs only), biocidal plant exudates and adsorption to organic matter. In addition, biological processes involved in microbial removal include predation, antibiotic interaction with other organisms, and natural die-off.[20]

The removal of pathogens related to chemical and biological processes which take place in the presence of macrophytes involves a series of complex interactions. As mentioned above, the rhizosphere is characterized by increased amounts of oxygen and other compounds which enhance biological activity. While some oxygen diffuses into to water column through the air-water interface, photosynthetic processes and oxygen leakage from plant roots contribute to oxygen availability as well.[19] Apart from oxygen, other compounds may be secreted from plant roots to fulfill various functions, including signaling and recruiting microbes that may be beneficial to their survival. The enhanced biological activity found in these vegetated systems enhances biodegradation of organics and adsorption of various contaminants, including pathogens, and is therefore an important characteristic of CWs.

In the case of treatment ponds, many of the processes described above are also important in the removal of contaminants. However, adsorption and filtration processes will be reduced due to a lack of significant plant coverage and contact with roots and other plant surfaces. On the other hand, such lack of vegetation increases solar exposure leading to enhanced photo-induced inactivation processes and, as a result, inactivation of microbes via solar processes may be the most important mechanism for microbial removal in treatment ponds.[21] Despite extensive research, there is still debate in the literature regarding the influence of various physico-chemical factors on inactivation and removal kinetics for both dark and solar-induced processes.[21,22] This uncertainty may be due to the fact that different microbes are inactivated by different mechanisms. Thus, models that take into account specific parameters (e.g. pH, dissolved oxygen) based on observed effects on one or more microbes of concern may yield highly inaccurate predictions when applied to other organisms. This points to the need for better understanding of the mechanisms and conditions that affect specific microbes (or at least classes of microbes) as the basis for the development of empirical models that better account for these fundamental processes.

Applications and concluding remarks

The results presented in this thesis contribute to the understanding of photochemical processes in NTS and other surface waters, regarding the formation of RI and inactivation kinetics *E. faecalis*. While the specific mechanisms and kinetics would vary in different contexts, this approach can better inform the prediction of microbial inactivation and indirect photolysis of other water constituents. Models have been developed to predict the removal of fecal coliform in treatment ponds that account for water depth[23] and the light attenuation,[24] acknowledging the importance of solar irradiance for the inactivation of this bacterial population. As more knowledge is gathered regarding the inactivation kinetics and mechanisms of other microbes, models can be modified (or developed) to account for a wider range of organisms. For example, trade-offs between HRT and depth (which are inversely proportional with constant area) can be better evaluated to achieve removal goals as more accurate removal kinetics are obtained. Specifically, in context where area is limited, HRT can be increased by increasing depth. This may be beneficial for those removal processes that do not rely as heavily on sunlight-driven processes, e.g. sedimentation, adsorption, but may affect disinfection more significantly. Furthermore, considerations for other parameters such land availability and cost may also be important components of design that require knowledge of how various processes are affected by depth.[25] Thus, a better understanding of the removal goals and the conditions required to achieve them would be of great use in optimizing NTS design.

In a broader sense, it is safe to assume that for the next few decades (i) water will continue to be a sought-after resource for many communities around the world, (ii) the development and capacity of large-scale, centralized wastewater treatment facilities will continue to lag behind population growth and wastewater generation in developing communities, and thus (iii) wastewater will continue to grow as a valuable source of water and nutrients that should be properly managed, treated, and (if possible) reused for irrigation or other purposes.

While the use of wastewater for irrigation emerged as a solution to the challenge of water scarcity, it brought with it its own set of challenges in the form of health and environmental risks. Thus, the promotion of relatively simple and inexpensive systems such as treatment ponds and wetlands to promote pathogen die-off and settlement of solids before wastewater reaches community members, crops, or is returned to the environment is very important. For instance, information, funding, and other resources and incentives can be presented to farmers and the broader community to encourage them to engage in safer practices.

With this in mind, wastewater management and reuse should be considered within the greater scheme of sustainability where the recovery of useful resources, i.e. water, nutrients, and organic matter, is encouraged in order to minimize resource extraction and optimize resource utilization. This addresses the issues of scarcity of resources as well as waste management while potentially improving the livelihoods of subsistence or commercial farmers and improving food security for their communities.

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Chapter 6 Conclusions

Concluding remarks

The work presented in this dissertation explored the photochemical processes involving organic matter (OM) in surface waters. It provides a better understanding of the fundamental interactions between sunlight and other water constituents and their implications in the performance and design of natural treatment systems (NTS), considered to be appropriate technologies for wastewater treatment, particularly in isolated or low-resource communities.

Quantifying the fluorescence and photosensitizing efficiency of organic matter shines light into the underlying mechanisms dictating its behavior. The absorbance of light by OM chromophores and subsequent formation of excited states leads to enhanced reactivity of these molecules and alters its interactions with its surroundings. Different pathways of energy relaxation will impact such interactions and thus affect a variety of environmentally relevant processes.[1–4] For example, excited states of OM can help degrade organic contaminants present in the environment or within treatment systems via direct interactions or through the formation of other reactive intermediates (RI) such as singlet oxygen ($^1O_2$) or hydroxyl radicals ($HO^\bullet$). Thus, a better understanding of these underlying processes provides insight into the behavior and impact of OM in the environment. It also allows for the establishing of relations between photochemical behaviors and optical properties of OM. As such, $^1O_2$ quantum yields and steady-state concentrations of different types of organic matter can be estimated based on relatively simple absorbance measurements.

The photochemical behavior of OM in natural systems also affects the concentrations of microbes in surface waters. In general, the dual role of OM as a light shield and photosensitizer must be understood to fully account for its impact on microbial populations. In the specific case of *Enterococcus faecalis* it is observed that the role of OM as a photosensitizer in the formation of RI leading to inactivation is greater than the inactivation decrease related to this shielding effect. While similar processes are expected to affect other microbes, these results cannot be widely generalized since the relative contribution of these processes may lead to overall increases or decreases in inactivation rates depending on the specific microbe and conditions being studied. Nonetheless, the accumulation of results for different microbial species and types does improve our overall understanding of the relevant mechanisms for inactivation in natural and engineered systems and should continue to be expanded. While results obtained in bench-scale experiments provide useful insights into relevant mechanisms, aided by more controlled conditions, validating and complementing these results in pilot- and full-scale systems will continue to improve our understanding of the most relevant factors affecting inactivation kinetics. This will ultimately improve the prediction of environmental processes and the design of treatment systems by accounting for the removal of pathogens and other constituents of concern.[5]

Furthermore, NTS should also be considered as options for the treatment of wastewater streams, particularly within low-resource communities, since they are less expensive to build and maintain than more sophisticated treatment systems, such as activated sludge. Beyond the immediate purpose of sanitation, such NTS should be thought of as part of broader water management practices that take into account the desires and needs of the communities they serve. For example, the use of treated streams for irrigation or other purposes may present additional benefits by enhancing water and nutrient recovery. It is also important to note that the potential applications of these systems are not limited to so-called developing countries, as they have been implemented in many industrialized countries as well.[6,7] Though certainly not exclusively, they would be particularly appropriate for more isolated or traditionally underserved communities within higher-income countries that would benefit from less expensive treatment options. Furthermore, the use of less energy- and chemical-intensive treatment options is in line with the broader goals of environmental sustainability that all societies (including those with higher income) should embrace. Thus, it makes sense that this type of treatment should be more broadly adopted in the so-called developed countries, not necessarily out of necessity but as part of efforts to minimize their environmental footprint.

Comments on hypotheses

I. Photochemical formation of $^1O_2$ is correlated to the optical properties and affected by molecular size and oxidation of WWOM

> *a) Decreasing MW leads to increased $^1O_2$ quantum yields.*
>
> *b) WWOM oxidation leads to increases in $^1O_2$ quantum yields*
>
> *c) $^1O_2$ quantum yields are positively correlated to the OM spectral slope and its multiplication by absorbance allows for estimations of $[^1O_2]_{SS}$*

Experiments were conducted to measure the quantum yields and steady-state concentrations of $^1O_2$ under simulated sunlight for various wastewater-derived samples, including size fractions and wastewaters undergoing oxidation. Higher quantum yields and E2:E3 values were observed for the smaller size fractions as well as those undergoing oxidation by both chlorine and ozone. Significant losses in absorbance were also observed for the oxidized samples, with O3 acting as a less selective oxidant. Generally, the quantum yield is positively correlated to the E2:E3 ratios, except for the highest oxidation doses. Multiplying this ratio, or the spectral slope, by the sample absorbance at a given wavelength (365 or 300 nm) results in values that are positively correlated to the measured $^1O_2$ steady-state concentrations.

II. Quantum yields of fluorescence and RIs increase concomitantly with decreasing MW of OM due to decreasing prevalence of non-radiative transitions

Fractionation of two distinct types of OM, namely Suwannee River natural organic matter (SRNOM) and treated wastewater effluent (BWW), was conducted and the photophysical and photochemical behaviors of these samples were studied. Similar trends emerged in both samples where fluorescence and photosensitizing efficiency in the formation of reactive intermediates ($^1O_2$, $HO^\bullet$, and ^3OM) increased with decreasing size. These trends can be explained by increases in radiationless decay (internal conversion) or other processes (e.g. charge transfer) with increasing size, which compete with fluorescence and intersystem crossing, leading to decreases in the quantum yields for fluorescence and the RI studied here.

III. Photoinactivation of enterococci is enhanced in NTS waters due to higher RI concentrations

a) Overall inactivation rates of Enterococcus faecalis increase in the presence of OM

b) The relative contribution of indirect exogenous photoinactivation increases with depth

Inactivation rates of *Enterococcus faecalis* were measured in sensitizer-free experiments (PBS) as well as in the presence of various types of organic matter under simulated sunlight. The sensitizer-free experiments displayed the lowest inactivation rates, pointing to the photochemical formation of RI as enhancing inactivation. While a portion of the light is absorbed by the OM in the samples (and thus prevented from reaching the microbes) the net effect is an increase in inactivation due to enhanced exogenous processes. Since the lower wavelengths are preferentially absorbed by OM and they are also the most effective in the direct and/or endogenous inactivation, the relative contribution of indirect exogenous processes increases with depth even as the overall rate decreases.

Further questions

While the photochemical behaviors of various OM types were evaluated through this work, there are additional questions that would justify further research and complement the results presented here. The wavelength dependence of the $^1O_2$ quantum yields was not established since all experiments were conducted using a polychromatic source (i.e. solar simulator). Previous studies have shown that the photosensitizing efficiency may increase with decreasing wavelength for certain samples, though weakly for many, and it is difficult to generalize this behavior across very distinct OM types.[8–12] Thus, studying this behavior in the context of wastewaters, OM size fractions, and other samples that have been less studied would contribute to a better understanding of this phenomenon. Such knowledge would have aided specifically in the development of the model presented in chapter 4 for estimating the contribution of exogenous inactivation processes as a function of depth as the photosensitizing efficiency of different wavelengths (which are absorbed to different) could be better accounted for.

Another relevant question that emerges during experiments relates to the specific parameters responsible for certain observed behaviors. Specifically, as OM samples are fractionated according to size, different photosensitizing efficiencies are measured. However, it is difficult to assess whether such differences are due to different sample compositions present in the different fractions or whether similar OM sub-structures are present but in smaller sized molecules. For instance, Fourier transform infrared spectroscopy could provide information regarding the relative prevalence of various bond structures. Comparing these data across samples could help determine how composition may be changing due to fractionation. More broadly, better characterization of the samples with respect to composition, size, and other characteristics would aid in understanding the underlying mechanisms that affect the observed bulk behaviors.

While the inactivation experiments pointed to the difference between endogenous and exogenous processes affecting bacterial survival, complementary analysis could provide more precise information regarding the inactivation mechanisms. For example, it is not clear however, whether exogenously produced RI can significantly affect the microorganism’s genes or if they act predominantly on other essential cell components, such as the membrane. While QPCR can help assess the DNA damage leading to inactivation on viruses, its applications would be more limited for bacterial inactivation. Thus, the development and application of new techniques that further this knowledge would be of great use. It would also be interesting to see how these various processes vary as a function of depth as well as investigating possible repair mechanisms after undergoing the different types of inactivating damage.

Finally, while models were developed to predict the $^1O_2$ steady-state concentration and the inactivation rates at different depths, this was done based on the results obtained under our specific experimental conditions. As such, this analysis could be complemented by pilot- or full-scale systems. For example, formation of 1O2 and inactivation kinetics could be evaluated within columns where the water depth can be varied, e.g. up to 1 meter. The role of particulate OM and other solids could also be evaluated by comparing the results of filtered vs. unfiltered samples. These conditions may better reflect those found in environmental and treatment systems and could then be validated by comparing results to the degradation rates of certain compounds or native microbial populations in full-scale systems. Ultimately, this improved knowledge should be translated into models and other means of informing the design of NTS to improve their performance, reliability, and acceptability.[5] This would lead to a better utilization of resources and optimization in the treatment of wastewater streams by expanding and improving the use of such systems.

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Appendix A. Supporting information: Singlet oxygen formation from wastewater organic matter

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The supplemental information contains 4 Figures.

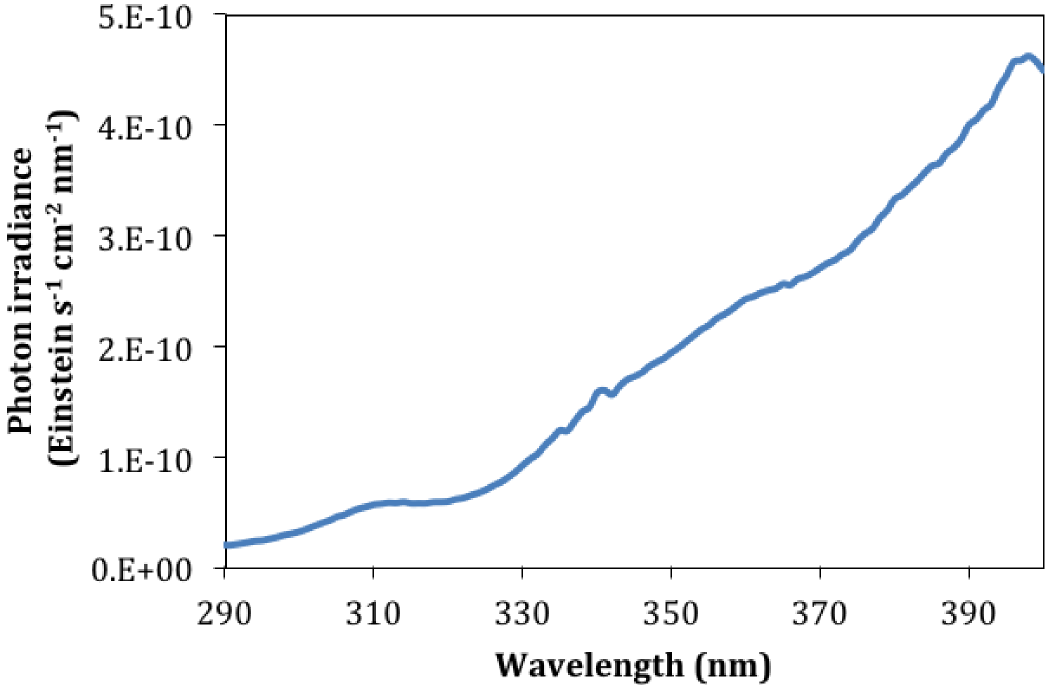

Figure A.1 Photon irradiance for the solar simulator used in exposure experiments, model Sol1A, Oriel Instruments (Stratford, CT) with 1000 W Xe lamp and 1.5 air mass filter. Irradiance was measured using an Ocean Optics spectrometer.

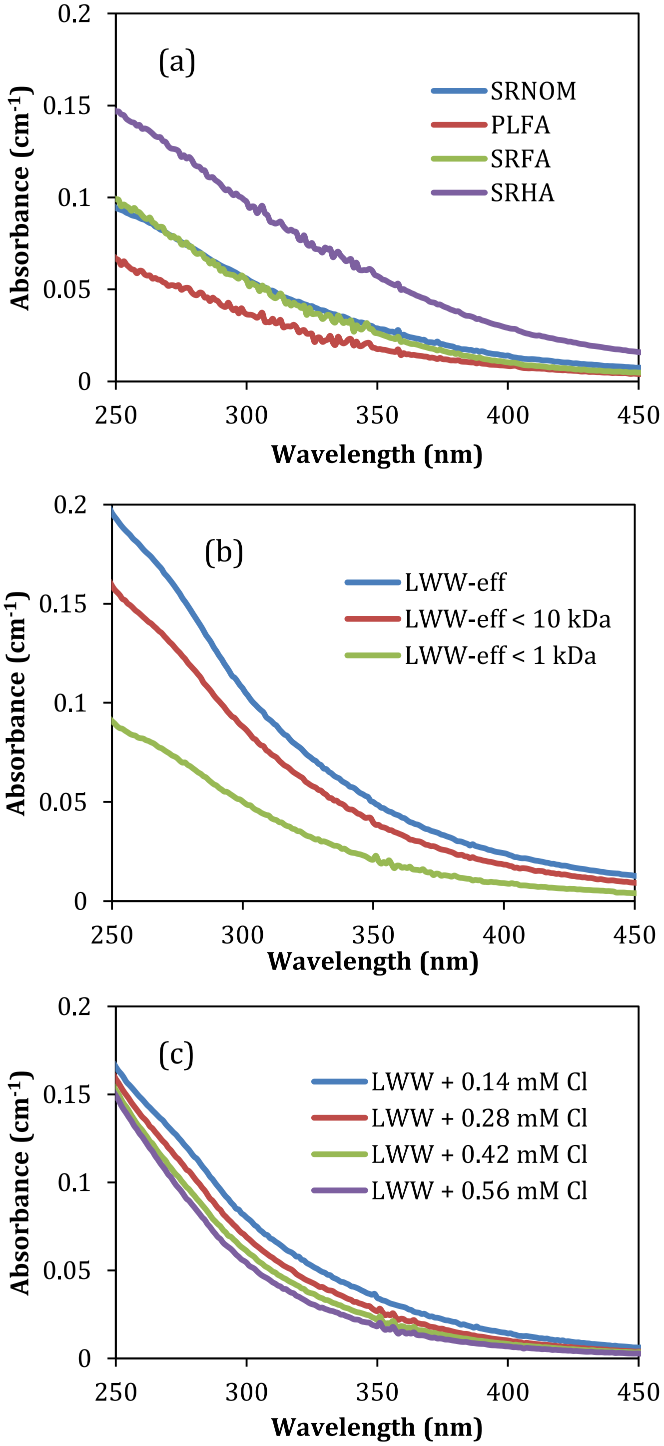

Figure A.2 Absorbance spectra (before exposure to solar simulator) for (a) IHSS isolates, (b) Louisville, CO wastewater effluent and fractions, and (c) Louisville, CO wastewater effluent with HOCl added.

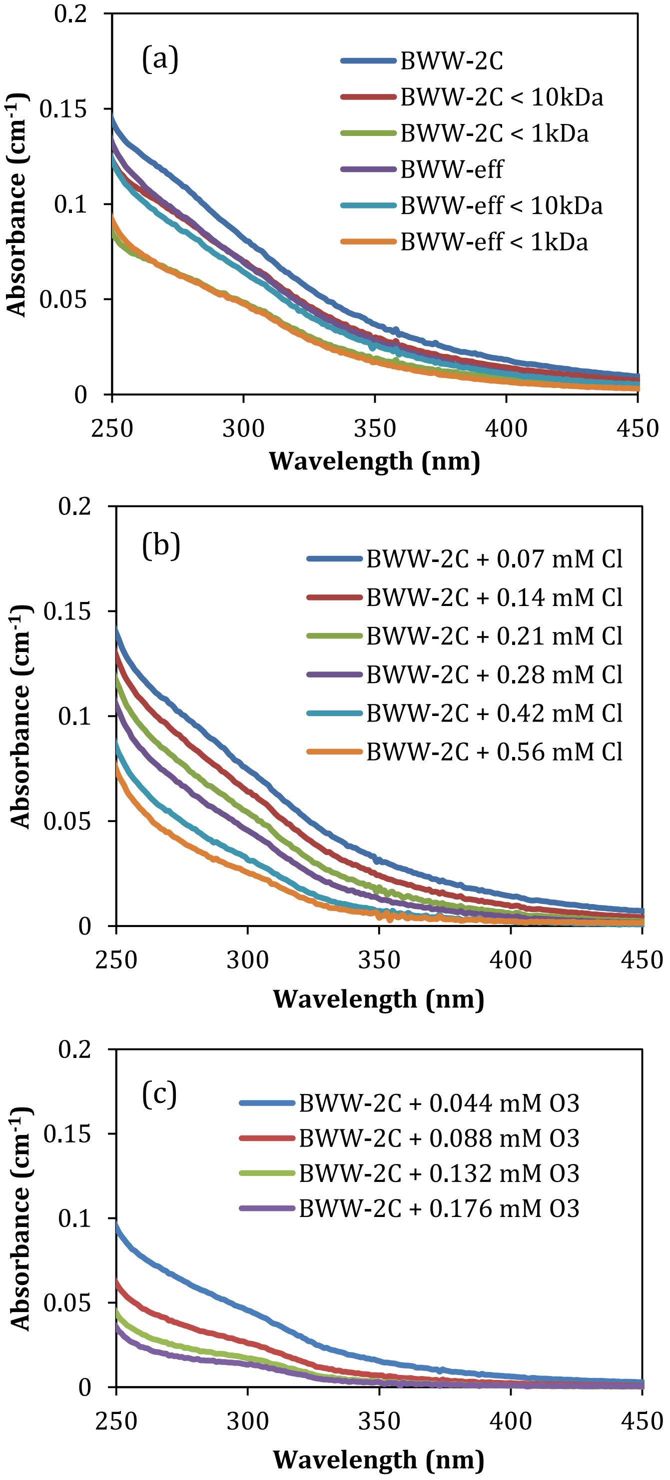

Figure A.3 Absorbance spectra (before exposure to solar simulator) for (a) Boulder, CO wastewater effluent and fractions, (b) Boulder, CO wastewater effluent with HOCl added, and (c) Boulder, CO wastewater effluent with O_3 added.

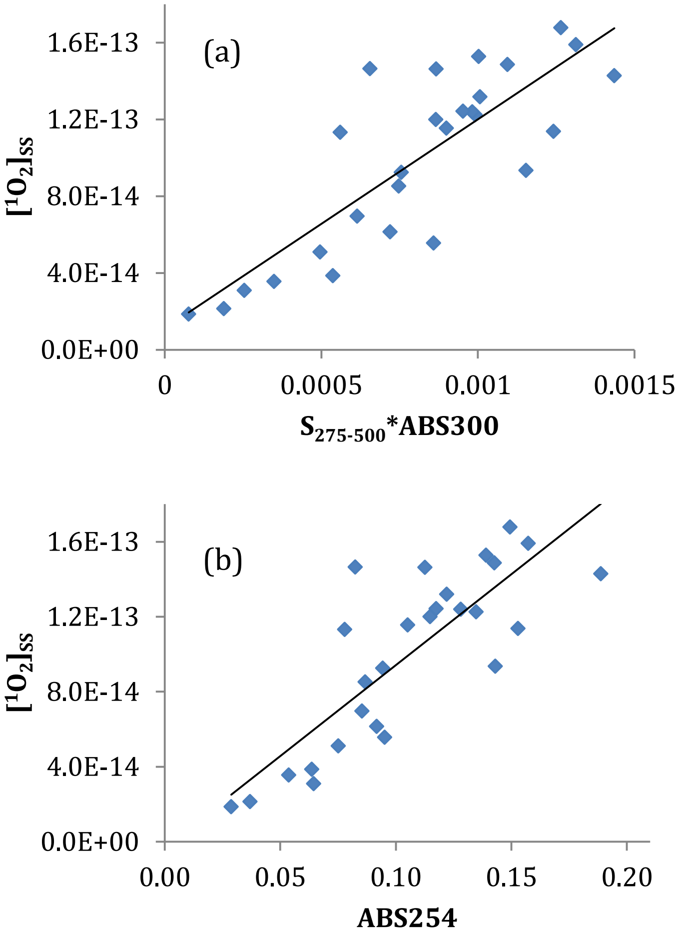

Figure A.4 $[^1O_2]_{SS}$ plotted against [$S_{275\text{-}500}$ x ABS300] (a) and ABS254 (or E2:E3 × ABS365) (b), with linear regressions, after subtraction of the contribution of nitrate to the total absorbance. Nitrate concentrations were 15.8 and 4.0 mg-N/L for BWW and LWW, respectively.

Appendix B. Supporting information: Relative impact of light screening and photosensitizers on *E. faecalis* inactivation in surface waters

The SI contains four figures showing *E. faecalis* inactivation rate as a function of dose (fluence); time required to reach 3-$\log_{10}$ inactivation as a function of $[^1O_2]_{SS}/Abs_{300}$; exogenous inactivation rates as a function of $[^1O_2]_{SS}$; and the screening factor and weighted screening factor as a function of depth for BC and BWW-1C.

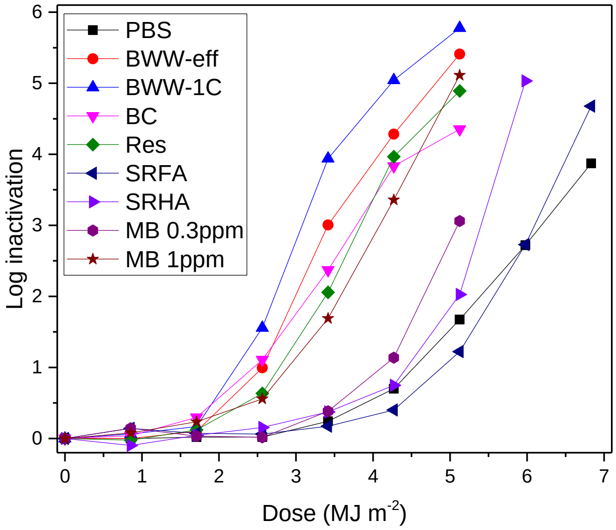

Figure B.1 ***E. faecalis*** **$\log_{10}$ inactivation as a function of applied simulated solar dose (fluence) calculated over wavelengths 290-600 nm.**

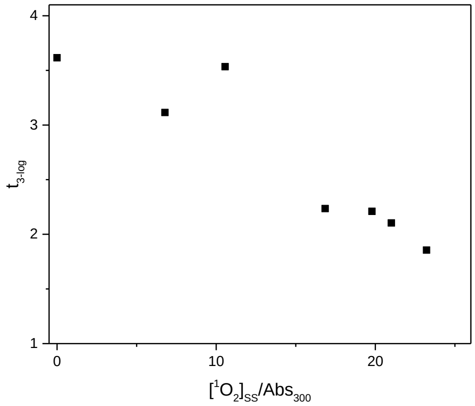

Figure B.2 Exposure time required to reach 3-$\log_{10}$ inactivation of *E. faecalis* as a function of the ratio of $[^1O_2]_{SS}$ and absorbance at 300 nm for each sample showing negative correlation ($P < 0.05$). Methylene blue data are excluded due to low absorbance

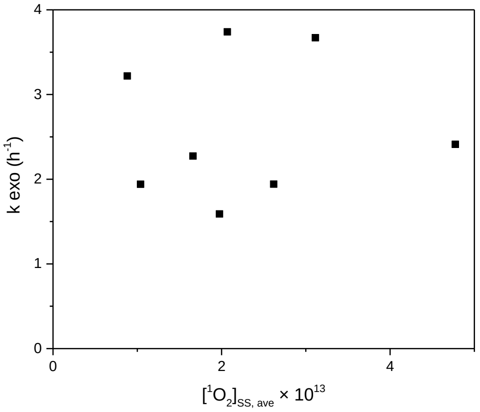

Figure B.3 Exogenous inactivation rate as function of [$^1O_2$]$_{SS}$, showing no significant correlation.

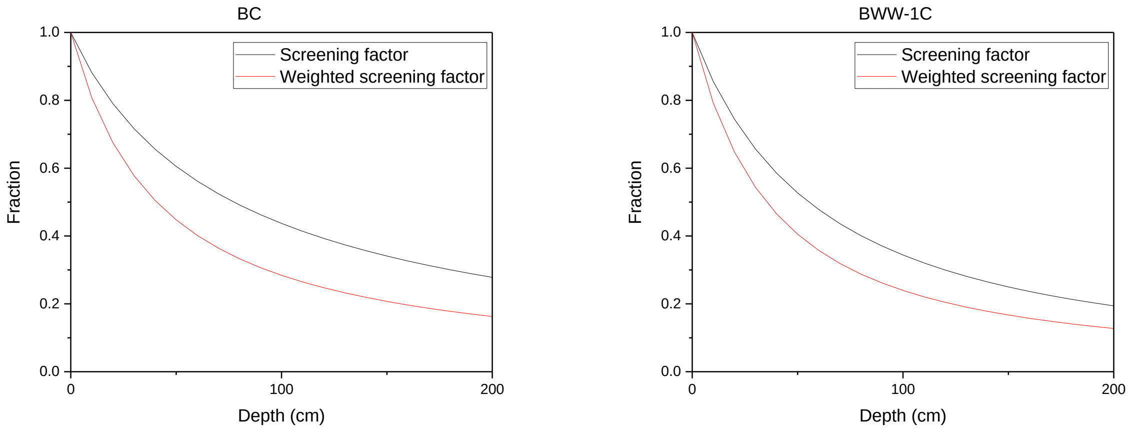

Figure B.4 Screening factor and weighted screening factor as a function of depth for BC and BWW-1C. WSF decreases more sharply than SF considering the photoinactivation action spectrum of *E. faecalis*.