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Ozone Degradation of Cyclophosphamide – Effect of Alkalinity and Key Effluent Organic Matter Constituents

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The influence of three effluent organic matter (EfOM) model compounds (alginic acid, peptone and natural organic matter-NOM) and alkalinity on the ozonation of cyclophosphamide (CPD) was investigated. The rate of ozone decay increased with increasing model compounds concentration in the order of peptone > NOM > alginic acid. Increasing alkalinity inhibited ozone decay at all concentrations of alginic acid and at low concentrations of NOM and peptone (DOC < 3 mg/L), while at high NOM and peptone concentrations the effect of alkalinity on ozone decay was minor. Presumably, ozone decay was mainly controlled by direct reaction with these two model compounds, resulting in •OH (hydroxyl radical) formation yield of 29% and 19%, for peptone and NOM respectively. In the presence of alginic acid •OH formation through a radical chain reaction resulted in a yield of 30%. Cyclophosphamide (CPD) removal decreased with increasing alkalinity and model compounds concentration (most pronounced for peptone and less pronounced for alginic acid); most likely due to increase in the scavenging effect on •OH.

Keywords Ozone, Hydroxyl Radicals, Cyclophosphamide, Alginic Acid, Peptone, NOM

INTRODUCTION

Ozone has potential in degrading and removing trace organic micropollutants in water (e.g., pharmaceuticals; e.g., Dodd et al. 2006; Wert et al. 2009). These organic contaminants are degraded during ozone application through two main pathways: direct oxidation by molecular ozone and the indirect radical oxidation, where •OH (hydroxyl radical) is the main contributor. The degradation kinetics of the contaminant and the contribution of each of the oxidation processes are

influenced by the target contaminant characteristics (i.e., its reaction rate with O_3 and •OH) and by the composition of the treated water.

Staehelin and Hoigné (1985) showed that the aqueous ozone is decomposed (and •OH formed) generally through a radical chain reaction initiated by reaction of O_3 with OH^-. This chain reaction can be promoted by solutes that transfer •OH into superoxide radical ion ($O_2^{•-}$; e.g., humic acid), or inhibited by solutes that do not promote $O_2^{•-}$ (e.g., carbonate). Alternatively, Pi et al. (2005) proposed that the radical chain reaction can be accelerated by different aromatic compounds, where *in-situ* hydrogen peroxide is a main intermediate and chain carrier; while Pocostales et al. (2010) suggested that •OH generation may additionally occur through ozone adducts to aromatic compounds, the elimination of singlet oxygen and the formation of phenol.

In natural waters, various researchers have identified natural organic matter (NOM) and alkalinity as the main promoters and inhibitors of the radical chain reaction in the ozonation process (e.g., Acero and von Gunten 2001). Huber et al. (2003) found that the ozonation efficiency of various pharmaceuticals, which react slowly with O_3 and quickly with •OH, increased with increasing NOM concentration and decreased with increasing alkalinity in different natural waters.

In wastewater effluent, ozonation kinetics differs from drinking waters due to the relatively high concentration and complexity of the effluent organic matter (EfOM). Effluent organic matter (EfOM) is mainly composed of NOM, soluble microbial products (SMPs) and non-biodegradable organic materials. SMPs (e.g., polysaccharides and proteins) mostly originate from the biological processes within the wastewater treatment plant (WWTP); while NOM, which includes a vast variety of organic molecules and functional groups, typically originates from the source water (i.e., drinking water; Shon et al. 2006). The relative contribution of the different EfOM constituents may vary with place, season and treatment.

Numerous studies have demonstrated the differences in ozonation kinetics of pharmaceuticals between natural water and wastewater effluents (e.g., Benitez et al. 2009). Zimmermann et al. (2011) investigated the degradation of different micropollutants in a full-scale reactor, treating secondary wastewater effluent, for ozone doses in the range of 0.21 to 1.24 g O_3: g DOC. They found that compounds reacting fast with ozone ($k_{P;O3} > 10^4$ l mol^{-1} s^{-1}) were eliminated at almost all O_3 doses, while substances with lower ozone reactivity ($k_{P;O3} < 10^4$ l mol^{-1} s^{-1}) were only fully eliminated at the high ozone doses.

Buffle et al. (2006) concluded that the decomposition of ozone in wastewater and the formation of •OH radicals are initiated and controlled by direct reaction of O_3 with reactive moieties in the EfOM, rather than through the radical chain reaction. Their study showed the presence of remarkably high •OH concentration during the first seconds of the O_3 process (higher than in most advanced oxidation processes (AOPs) in natural waters).

In wastewater effluents, it is difficult to model ozone degradation of micropollutants as a result of the indistinct effects of different EfOM compounds. The objective of the present study is to determine the degradation of the anticancer drug cyclophosphamide (CPD) by ozonation at, either low or high alkalinity values, in the presence of different EfOM's constituents. Cyclophosphamide (CPD) degradation is of particular interest due to its known mutagenic properties and its resistance to most conventional and advanced wastewater treatments (Kim et al. 2008).

Three model compounds were used to simulate main constituents in the EfOM, separately and in a mixture: (I) Alginate, an acidic polysaccharide, extensively studied in the field of biofouling in water and wastewater systems, and a potential contributor to SMPs in wastewater effluent (Lee et al. 2006), (II) Peptone from casein, used as a source of amino acids and peptides and, (III) Suwannee River NOM, used as a NOM contributor.

MATERIAL AND METHODS

Standards and Reagents

Cyclophosphamide (CPD) standard (>99% purity) was obtained from Sigma-Aldrich, LC-MS grade acetonitrile, methanol and water from Bio-Lab Ltd. (Jerusalem, Israel). Cyclophosphamide (CPD) stock solution was prepared by dissolving the compound in deionized (DI) water (Direct-Q3 UV system, Millipore-France; resistivity > 18 mΩ cm) at a concentration of 100 mg/L. The probe compound p-chlorobenzoic acid (pCBA) was used to determine •OH radical reaction rate constants with the EfOM model compounds (Cat. No. 13,558-5, Sigma Aldrich, Germany).

Alginic acid (alginate), extracted from brown algae, was purchased from Sigma-Aldrich (CAS: 9005-32-7). Peptone from casein was obtained from Fluka (CAS: 91079-40-2). Suwannee River NOM was obtained from the International Humic Substances Society-IHSS (St. Paul, MN). Alkalinity of the water was modified using sodium bicarbonate (Sigma-Aldrich). Stock solutions of alginic acid and NOM (500 mg/L) were prepared in DI water, adjusted to pH 10 by the addition of NaOH. Peptone (500 mg/L) and sodium bicarbonate (30 g/L) stock solutions were prepared in DI water. All stock solutions were filtered through a 0.45 μm cellulose acetate filter.

Cyclophosphamide Ozonation at Different EfOM Model Compounds Solutions

Ozone experiments were performed in a thermostated (21 °C) 1-L glass cylindrical batch reactor (Ace-glass, Vineland, NJ). Ozone was generated from pure oxygen (>99.9%) using an ozone generator (2–5 g/h, OZO-1VTT, Ozomax, Canada). The ozone stream was introduced into the aqueous solution using a diffuser located at the bottom of the reactor, while another tube carried off gases from the headspace of the reactor to the ozone destructor. The tested solution was constantly mixed via magnetic stirring. Ozone in the aqueous solution was continually measured using the WADIS310 dissolved O_3 sensor (Walchem Corporation, Holliston, MA).

Cyclophosphamide (CPD) ozonation experiments were designed to examine the influence of the following parameters: (a) different EfOM model compounds at dissolved organic carbon-DOC concentration range of 0–8 mg/L as C and, (b) the influence of alkalinity at concentrations of 25 and 200 mg/L as $CaCO_3$ (referred in the text as low and high alkalinity). In all cases, experiments were initiated by diffusing the ozone stream into a solution composed of phosphate buffer (2.5 mM) at pH 7.6 (within the pH range of wastewater effluent) and different concentrations of sodium bicarbonate, until approximately 5.3 mg/L dissolved O_3 concentration was achieved.

Ozone diffusion was then stopped, followed by the injection of CPD (initial concentration of ∼1 mg/L) and different concentrations of the tested EfOM model compounds. Sampling began after a mixing time of ∼10 s, when dissolved O_3 concentration reached exactly 5 mg/L. Samples (2 mL) were taken periodically, quenched immediately with excess of sodium thiosulfate to decompose residual O_3 and analyzed chromatographically for CPD concentration. Although typical mg O_3:mg DOC ratio do not exceed 1, in the present experiments ozone dose was held constant in order to measure the isolated effect of different DOC and alkalinity concentrations. The pH of the solution remained steady throughout the experiment duration.

Determination of the EfOM Model Compounds Rate Constants with •OH

The second-order rate constants of •OH with the EfOM model compounds ($k_{EfOM,•OH}$, L/(molC) s) were determined using a modified UV/H_2O_2 method adapted from Rosenfeldt and Linden (2004). Experiments were conducted in a

quasi-collimated beam apparatus equipped with a 0.45 kW polychromatic (200–300nm) medium-pressure (MP;Ace-Hanovia Lamp Cat. No. 7830-61; from Ace Glass, Inc.) Hg vapor lamp (described in details elsewhere; Lester et al., 2010). UV-based AOP was used in the present study as a simple method for obtaining the rate constants with •OH. Irradiation experiments were performed on buffered water samples (100 mL PBS 2.5 mM at pH 7.6) with added pCBA (1 µM), H_2O_2 (1.47 mM) and different concentrations of the tested EfOM model compound (0–8 mg/L as C). The UV incident irradiance was obtained using a calibrated spectroradiometer (USB4000, Ocean Optics, Florida, USA) and the UV absorbance of the treated solution was measured via UV-Vis spectrophotometer (Varian, Cary 100BIO, Victoria, Australia).

Determination of •OH Yield

•OH yield in the ozonation process was determined using t-butanol (Nöthe et al. 2009), a known •OH radical scavenger ($k_{•OH,t\text{-butanol}} = 6 \times 10^8$ M^{-1} s^{-1}). In this method O_3 is added to samples containing an EfOM model compound (5 mg/L as C) and t-butanol in large excess (100 mM). Under these conditions almost all formed •OH radicals react with t-butanol to generate formaldehyde (among other products) at a yield of ~50% (Flyunt et al. 2003). Concentration of formaldehyde was measured using the Nash method (Nash 1953).

Analytical Methods

Cyclophosphamide (CPD) was detected and quantified by HPLC-UV Agilent, model 1100 (ACE-RP C18 column 2.5 mm × 250 mm) and an MS detector (Finnigan LCQ). The mass spectrometer was used in positive electro-spray ionization (ESI) mode and the probe temperature was set to 220

°C. The flow from the HPLC was passed through a split connector with 60 µL/min of effluent introduced into the MS interface. Ions in the range 200–300 m/z were registered in the conventional scanning mode.

The mobile phase was ammonium formate 0.05M (A) and methanol (B), at pH 5. The mobile phase eluent gradient started with 50% of eluent A, followed by a 2.5-min linear gradient to 30% of eluent A, 3-min isocratic elution and a 2 min linear gradient back to 50% of eluent A, maintained for 4 min to equilibration time. Conditions for pCBA quantification are detailed in Lester et al. (2010). Dissolved organic carbon (DOC) of the EfOM solutions was measured using a TOC analyzer (Apollo 9000, Tekmar Dohrmann, Mason, OH).

RESULTS AND DISCUSSION

O_3 Decomposition

The difference in dissolved ozone decomposition in the presence of the EfOM model compounds, at low and high alkalinity values, is demonstrated in Figure 1, where $Ln [O_3]_t/[O_3]_0$ is plotted with time for alginic acid, NOM and peptone at DOC concentration of 1 mg/L (as C), and alkalinity concentrations of 25 and 200 mg/L. In the presence of NOM and peptone, an initial rapid O_3 decomposition phase was observed (0–60 s), followed by a second slower decomposition phase.

Nöthe et al. (2009) observed three-phase kinetics for ozone decay in wastewater effluent, where the first phase occurred within seconds. Therefore, we suspect that the two phases measured in our study represent the latter two phases of a three-phase O_3 decay kinetics. In contrast, only one O_3 decomposition phase was observed in the presence of alginic
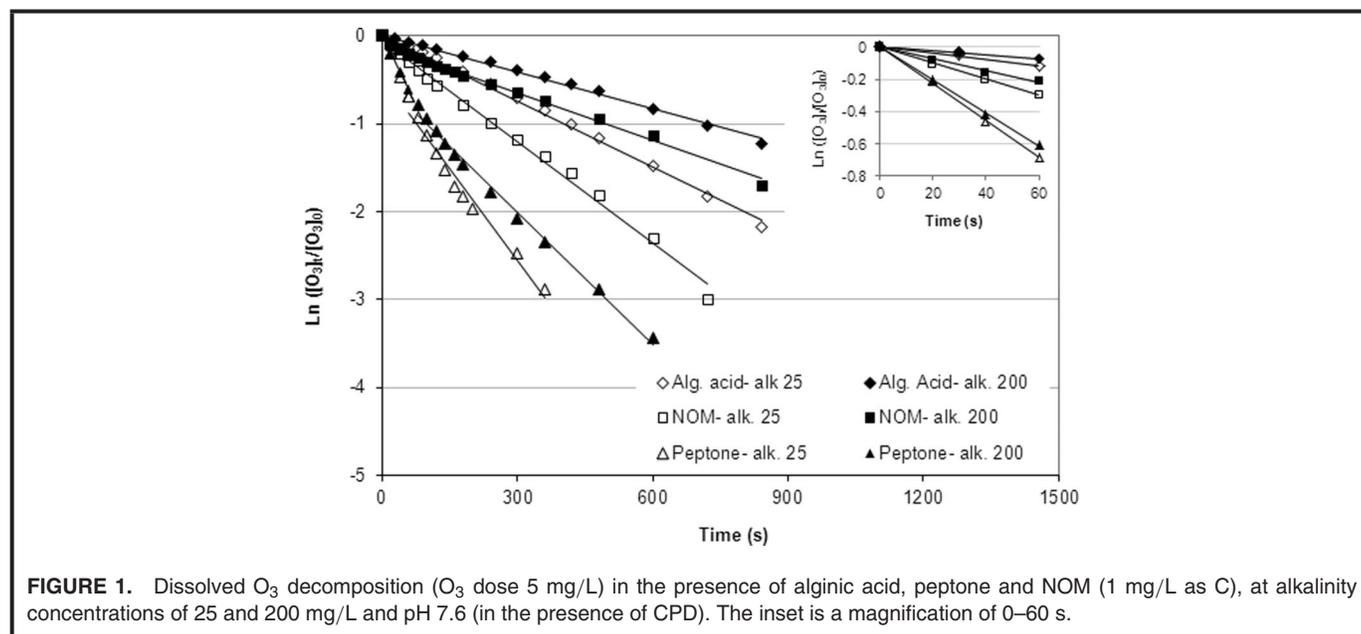

FIGURE 1. Dissolved O_3 decomposition (O_3 dose 5 mg/L) in the presence of alginic acid, peptone and NOM (1 mg/L as C), at alkalinity concentrations of 25 and 200 mg/L and pH 7.6 (in the presence of CPD). The inset is a magnification of 0–60 s.

TABLE 1. First-Order Ozone Decay Rate Constant $k*(k_{sec}**)$, s^{-1}

	TOC mg L^{-1}					
	0	0.5	1	3	5	8
Alkalinity 25 mg L^{-1}						
Alginic acid	0.0026	0.0027	0.0026	0.0039	0.0065	0.0081
NOM	0.0028	0.0036	0.0050	0.0135	0.0210	0.0380
		(0.0029)	(0.0034)	(0.0115)	(0.0161)	(0.0205)
Peptone	0.0025	0.0076	0.0125	0.0288	0.0357	0.0407
		(0.0047)	(0.0068)	(0.0173)	(0.0190)	(0.0204)
Mixture	0.0025	0.0059	0.0089	0.0198	0.0280	0.0373
		(0.0043)	(0.0053)	(0.0172)	(0.0218)	(0.236)
Sum***	0.0026	0.0046	0.0070	0.0158	0.0211	0.0271
		(0.0033)	(0.0044)	(0.0108)	(0.0139)	(0.0155)
Alkalinity 200 mg L^{-1}						
Alginic acid	0.0016	0.0015	0.0014	0.0017	0.0024	0.0024
NOM	0.0014	0.0025	0.0037	0.0125	0.0193	0.0362
		(0.0016)	(0.0017)	(0.0062)	(0.0109)	(0.0172)
Peptone	0.0014	0.0049	0.0103	0.0282	0.0351	0.0391
		(0.0018)	(0.0049)	(0.0161)	(0.0173)	(0.0189)
Mixture	0.0014	0.0037	0.0058	0.0155	0.0254	0.0344
		(0.0018)	(0.0021)	(0.0111)	(0.0180)	(0.0194)
Sum***	0.0015	0.0031	0.0054	0.0145	0.0187	0.0234
		(0.0016)	(0.0029)	(0.0084)	(0.0101)	(0.0120)

*k_{sec}– First-order rate constant for the first (rapid) phase of O_3 decay.
**k_{sec}– First-order rate constant for the second (slower) phase O_3 decay.
***Sum $= k_{alginic\ acid}$x $0.4 + k_{NOM} \times 0.2 + k_{Peptone} \times 0.4$.

acid. In all cases, ozone decay exhibited an apparent first-order kinetics, and generally, in the order of: peptone > NOM > alginic acid (Figure 1). The O_3 first-order decay rate constants in the presence of the EfOM model compounds, for the rapid and slower phases, are presented in Table 1 (k, 1/s). O_3 decay in the absence of the EfOM model compounds (control experiments) exhibited an expected slow one-phase kinetics (Staehelin and Hoigne 1982), as shown in Figure 2.

The difference in ozone decomposition rate between the EfOM model compounds depends on their structure and, more specifically, on the existence of different moieties reacting directly with ozone and/or promoting the radical chain reaction, as follows:

- NOM structure is highly complex, consisting of a wide variety of organic molecules (e.g., humic acids) and functional groups (e.g., phenolic moieties). Numerous studies have already confirmed the reactivity of NOM and its influence on ozone decomposition (e.g., Westerhoff et al. 1999). For example, Mvula and von Sonntag (2003) showed that phenol may enhance O_3 decomposition both by direct reaction and by promoting the radical chain reaction.
- Peptone contains a mixture of small proteins, peptides and amino acids. Buffle and von Gunten

(2006) have demonstrated the high reactivity of deprotonated amino compounds in general, and of different amino acids in particular toward ozone. Moreover, Hoigné and Bader (1983b) found extremely high rate constants for the reaction of ozone with different amino acids which contain thio groups (e.g., cysteine). The peptide linkage connecting the α-amino group of the amino acids in polypeptides and proteins has a very low reactivity toward ozone (Pryor et al. 1984). Therefore, the high decomposition rate of ozone in the present study is probably due to its direct reaction with free amino acids and reactive side-chain groups in the peptides and proteins.
- Alginic acid is a linear polysaccharide composed of mannuronic and guluronic acid subunits. In general, saturated compounds such as polysaccharides react slowly with molecular ozone (e.g. reaction rate constant of ozone with glucose = 0.45 M^{-1} s^{-1}; Hoigné and Bader 1983a), thus the radical chain reaction will most likely predominate O_3 decay (hence the one-phase kinetic). Akhlaq et al. (1990) concluded that over 70% of the •OH radicals reacting with alginic acid during ozonation lead to the formation of O_2•$^-$, further reacting with O_3 to enhance its decay.

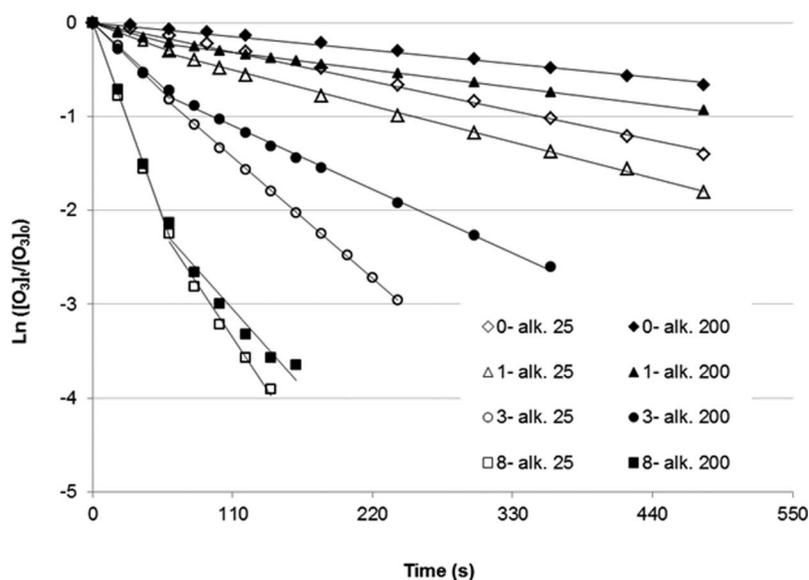Y. Lester, D. Avisar, and H. Mamane   March–April 2013

FIGURE 2. Dissolved O_3 decomposition (O_3 dose 5 mg/L) in the presence of different NOM concentrations (as DOC), at different alkalinity concentrations of 25 and 200 mg/L and pH 7.6 (in the presence of CPD). Numbers in the legends (0, 1, 3 and 8) refer to DOC concentration in mg/L.

The influence of the EfOM compound's concentration on ozone decomposition is demonstrated in Figure 2, for NOM at DOC values of 0, 1, 3 and 8 mg/L, and alkalinity concentrations of 25 and 200 mg/L, and in Table 1. Generally, as expected, ozone decay rate increases with increase in DOC concentrations for all EfOM model compounds at both low and high alkalinity values. However, in the presence of alginic acid, the increase in ozone decay rate initiates only at DOC > 1 mg/L, emphasizing the alginic acid's low reactivity toward molecular ozone.

Carbonate alkalinity is known to inhibit ozone decomposition in water by reacting with the generated •OH radicals, and forming carbonate radicals ($CO_3^{•-}$) that do not react directly with ozone nor promote the radical chain reaction (Staehelin and Hoigné 1982).

Reaction rates of $CO_3^{•-}$ with organic compounds are usually several orders of magnitude lower than reaction rates of •OH (Huang and Mabury 2000), therefore their influence on CPD removal in also minor. The stabilizing effect of alkalinity on ozone was demonstrated by comparing ozone decay rate at low and high alkalinity for a specific EfOM model compound concentration (Table 1). For alginic acid, the stabilizing effect of alkalinity on O_3 decay rate was clearly demonstrated throughout the entire DOC range, where increasing alkalinity at a specific DOC value always decreased ozone decay rate by more than 10%. In the presence of NOM, alkalinity inhibition was less pronounced during the first phase of O_3 decay, for DOC ≥ 3 mg/L. For peptone, the effect of alkalinity was minor for DOC ≥ 3 mg/L, during both first and second O_3 decay phases.

The different effects of alkalinity on O_3 decay emphasize the diverse mechanisms responsible for O_3 decay in the presence of the different EfOM model compounds. Using alginic acid, ozone decay occurred most likely through the radical chain reaction path. This reaction path is relatively slow and highly sensitive to the presence of •OH scavengers (i.e., alkalinity). In solutions containing the highly reactive NOM and peptone, decomposition of ozone followed both the direct and radical chain reaction paths.

Direct reaction of ozone with different moieties in the organic matter may be more rapid and independent of alkalinity concentration (Buffle et al. 2006). The relative contribution of each path differs in the first and second phase of O_3 decay and, depends on the EfOM model compound's concentration and its reactivity. For example, direct reaction of O_3 with the organic matter is probably the dominant mechanism for O_3 decay in the presence of NOM, during the first O_3 decay phase, at DOC ≥ 3 mg/L and, in the presence of peptone, at both the first and second O_3 decay phases, at DOC ≥ 3 mg/L.

In an attempt to better simulate "real" wastewater effluent, O_3 decomposition experiments were conducted using a mixture of the model compounds. Since the fraction distribution of EfOM may vary substantially depending on the wastewater origin and the type of treatment (Imai et al. 2002; Jarusutthirak et al. 2002), an exemplary ratio of 2:1:2 (for alginic acid:NOM:peptone) was chosen. The experimental O_3 decomposition rate (termed mixture) was compared to the calculated value (termed sum; i.e., weighted sum of the individual rate constants; Table 1). The experimental rate

constants were higher than the calculated values in almost all cases. Rosario-Ortiz et al. (2008) found that the reaction rate constants of •OH with different non-isolated EfOM were 3–5 times higher than the rate constants of •OH with fractionated EfOM. Thus, a system containing various organic solutes is not a simple mixture with respect to its reactivity toward ozone and •OH. Possibly, interactions between the examined EfOM model compounds contributed to this phenomenon.

Cyclophosphamide (CPD) Removal

The removal of CPD was recorded until all applied ozone was consumed (i.e., 5 mg/L); however, under different experimental conditions, CPD concentration decreased below the HPLC-MS limit of detection (100 ng/L). Therefore, CPD removal is presented herein for an applied ozone dose of only 3 mg/L (i.e., until dissolved ozone reached 2 mg/L). All CPD removal experiments were duplicated and relative standard deviations were less than 10% in all cases. Figure 3 presents CPD removal as a function of DOC concentration, for the different EfOM model compounds and alkalinity concentrations, at an applied ozone dose of 3 mg/L.
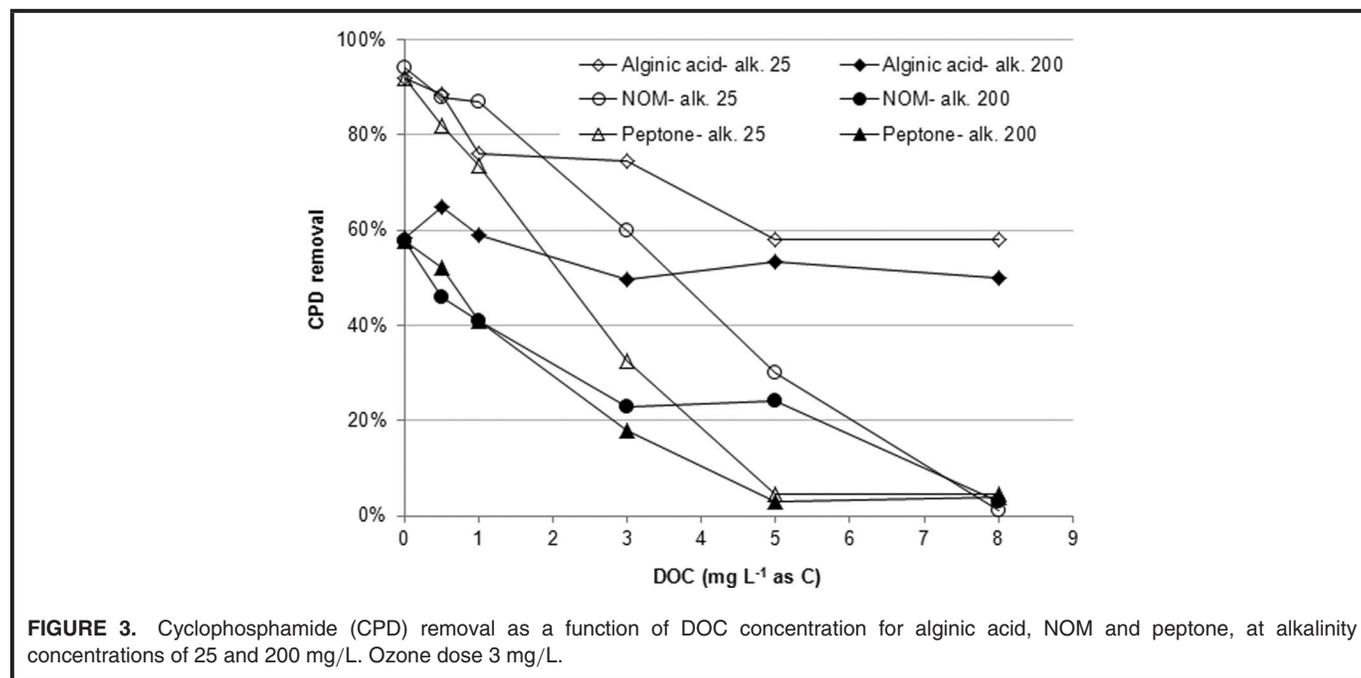
Cyclophosphamide (CPD) removal in a buffered water (without organic matter, DOC = 0 mg/L in Figure 3), for low and high alkalinity levels was approximately 92% and 58%, respectively. Generally, CPD removal decreased with increase in DOC concentration (with an exception of alginic acid at high alkalinity and DOC 0.5 mg/L), and in some cases it reached a plateau at higher DOC values. The influence of peptone on CPD removal was most pronounced, where almost no CPD removal can be seen at DOC $\geq$ 5 mg/L; while in the presence of alginic acid, CPD removal decreased only moderately with increase in DOC concentration.

Due to the low reaction rate of CPD with molecular ozone ($k_{O3,CPD} = 2.8$ M^{-1} s^{-1}; Lester et al. 2011), its removal during the ozonation process occurs mainly through oxidation by •OH radicals ($k_{•OH,CPD} = 1.3 \times 10^9$ M^{-1} s^{-1}; Lester et al. 2011). Thus, the differences in the model compounds' impact on the CPD removal can be explained by differences in the •OH production yield and different scavenging effects on •OH radicals by the EfOM model compounds (evaluated in the following sections).

Higher CPD removal is obtained at low alkalinity values compared to high alkalinity values for all EfOM model compounds at DOC concentration < 5 mg/L, due to the scavenging effect of alkalinity on •OH radicals. At higher DOC levels ($\geq$5 mg/L), a comparable CPD removal rate can be seen at both alkalinity values, indicating that the scavenging effect of the EfOM model compounds is dominant. In natural waters, alkalinity is considered a main •OH scavenger; while in water with high concentration of organic matter (i.e., wastewater effluent), its contribution to the water scavenging is less significant. Elovitz et al. (2000) estimated the •OH scavenging of alkalinity in lake water to be approximately 50% of DOC scavenging; while Nöthe et al. (2009) have calculated this value to be ~10% in wastewater effluent.

Rate Constant for the Reaction of •OH with the EfOM Model Compounds

UV/H$_2$O$_2$ degradation of pCBA, in the presence of different concentrations of the EfOM model compounds, was used to calculate $k_{•OH,EfOM}$. Degradation of pCBA involves direct UV photolysis and indirect photo-oxidation by •OH radicals, as described in the following equations (Rosenfeldt and Linden 2004):

FIGURE 3. Cyclophosphamide (CPD) removal as a function of DOC concentration for alginic acid, NOM and peptone, at alkalinity concentrations of 25 and 200 mg/L. Ozone dose 3 mg/L.

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$$-\ln \frac{[pCBA]_t}{[pCBA]_0}/t = k_{obs} = k' + k_{pCBA,\bullet OH}\,[\bullet OH]_{ss} \quad [1]$$

$$k' = \phi_{pCBA} \times k_{s,pCBA} \quad [2]$$

$$k_{s,pCBA} = \sum_{200-300} \frac{10^{-3} \times E_p^0(\lambda)\varepsilon_{pCBA}(\lambda)\left[1 - 10^{-a(\lambda)z}\right]}{a(\lambda)z} \quad [3]$$

where, $[pCBA]_0$ and $[pCBA]_t$ are initial pCBA concentration (M) and its concentration after exposure time t (s), k_{obs} and k' are the observed (total) and direct-photolysis time-based pseudo-first-order degradation rate constants of pCBA respectively (1/s). $[\bullet OH]_{ss}$ is the steady-state $\bullet OH$ radical concentration (M) and $k_{pCBA,\bullet OH}$ is the second-order rate constant of pCBA reaction with $\bullet OH$, reported to be 5×10^9 M^{-1} s^{-1} (Buxton et al. 1988). Φ_{pCBA} is the quantum yield for pCBA removal (0.0182 mole/Einstein; Lester et al. 2010), $k_{s,pCBA}$ is the specific rate of light absorption by pCBA (Einstein /mole s). $E^0p(\lambda)$ is the incident photon irradiance (Einstein/cm^2 s), $\varepsilon_{pCBA}(\lambda)$ is the molar (decadic) absorption coefficient of pCBA (1/M cm), $a(\lambda)$ is the solution absorption coefficient (1/cm) and z is the depth of solution (cm).

The steady-state concentration of $\bullet OH$ radical was calculated as the ratio of the formation of $\bullet OH$ radicals to the destruction of the radicals (Rosenfeldt and Linden 2004):

$$[\bullet OH]_{ss} = \frac{R_{\bullet OH}^{Form}}{k_{pCBA,\bullet OH}[pCBA] + k_{H_2O_2,\bullet OH}[H_2O_2] + k_{EfOM,\bullet OH}[EfOM]} \quad [4]$$

where, $k_{H_2O_2,\bullet OH}$ (M^{-1} s^{-1}) and $k_{EfOM,\bullet OH}$ (L/(molC) s) are the second-order rate constants of $\bullet OH$ with H_2O_2 and the EfOM model compound respectively. $R_{\bullet OH}^{Form}$ is the rate of

$\bullet OH$ formation (M/s) and is calculated using Equation [5], taking into account the UV light absorbance of the irradiated solution.

$$R_{\bullet OH}^{Form} = k_{s,H_2O_2} \times \phi_{H_2O_2} \times [H_2O_2] \quad [5]$$

where, $\phi_{H_2O_2}$ is the quantum yield for $\bullet OH$ formation (QY = 1; Baxendale and Wilson 1957), and k_{s,H_2O_2} is the specific rate of light absorption by H_2O_2, calculated using Equation [3] (modified for H_2O_2).

Substituting Equation [4] into [1], inverting both sides and rearranging results in Equation [6].

$$\frac{R_{\bullet OH}^{Form}}{k_{obs} - k'} = [pCBA] + \frac{k_{H_2O_2,\bullet OH}[H_2O_2]}{k_{pCBA,\bullet OH}} + \frac{k_{EfOM,\bullet OH}[EfOM]}{k_{pCBA,\bullet OH}} \quad [6]$$

Figure 4 is a plot of $R_{\bullet OH}^{Form}/k_{obs} - k'$ vs. the EfOM model compounds' concentration. Multiplying the slope of the linear lines by the known value of $k_{pCBA,\bullet OH}$ resulted in reaction rate constants of $\bullet OH$ with alginic acid, NOM and peptone of 0.92×10^8, 0.95×10^8 and 1.30×10^8 L/(molC) s, respectively. The calculated values are lower than most values presented in the literature for EfOM constituents. Moreover, the similar values obtained for alginic acid and NOM are unexpected, in view of the differences in their characteristics (aliphatic vs. aromatic). The Suwannee River NOM used in our study contains however relatively high proportion of polar aliphatic substances in addition to humic and fulvic substances (Serkiz and Perdue 1990), which may partially explain its relatively low reaction rate constants with $\bullet OH$.

Westerhoff et al. (1999), using ozone as an $\bullet OH$ source, found $\bullet OH$ reaction rate constants with Suwannee River
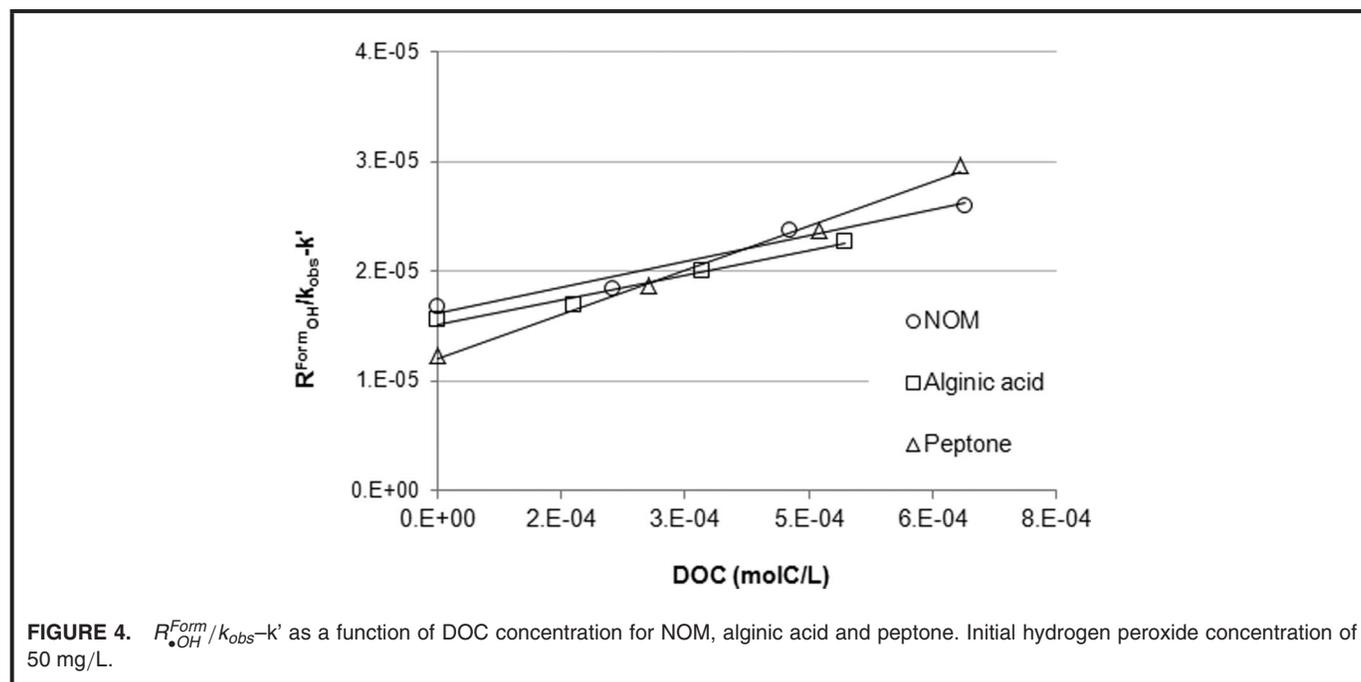

FIGURE 4. $R_{\bullet OH}^{Form}/k_{obs} - k'$ as a function of DOC concentration for NOM, alginic acid and peptone. Initial hydrogen peroxide concentration of 50 mg/L.

humic and fulvic acids to be 8.1 and 3.7 $\times$ 10^8 L/(molC) s respectively. A later study by Westerhoff et al. (2007), using electron pulse radiolysis, presented values of 1.6 $\times$ 10^8 L/(molC) s for the reaction of •OH with Suwannee River fulvic acids, and an average value of 2.23 $\times$ 10^8 L/(molC) s for seven DOM isolates from different sources. Myint et al. (1987) measured the rate constant for the reaction of •OH with hyaluronic acid (a carbohydrate polymer with some resemblance to alginic acid) to be 7$\times$10^8 M^{-1} s^{-1}, expressed in terms of the disaccharide repeating sub-unit (equivalent to approximately 3$\times$10^8 L/(molC) s), using pulse radiolysis.

The reaction rate constants for peptone and alginic acid with •OH obtained in the present study correlate well with the different CPD removal behaviors (Figure 3). Increasing the concentration of the highly •OH reactive peptone results in a sharp decrease in CPD removal rate, while addition of the less-reactive alginic acid only moderately decreases the CPD removal rate. For NOM, the low reaction rate constant with •OH may provide an explanation only if considering that a high portion of the •OH radicals reacting with NOM are scavenged (i.e., do not promote the chain reaction by producing $O_2^{•-}$).

•OH Yield of Formation

The •OH concentration (calculated through the generation of formaldehyde) for different O_3 doses in the presence of the EfOM model compounds is presented in Figure 5. From the slope of the linear lines the yield for •OH formation in the presence of alginic acid, NOM and peptone was found to be approximately 30%, 19% and 29%, respectively. The calculated yields are higher than a value previously calculated by Nöthe et al. (2009) for the ozonation of wastewater effluent ($\sim$13%), but closer in value to a yield found by Mvula
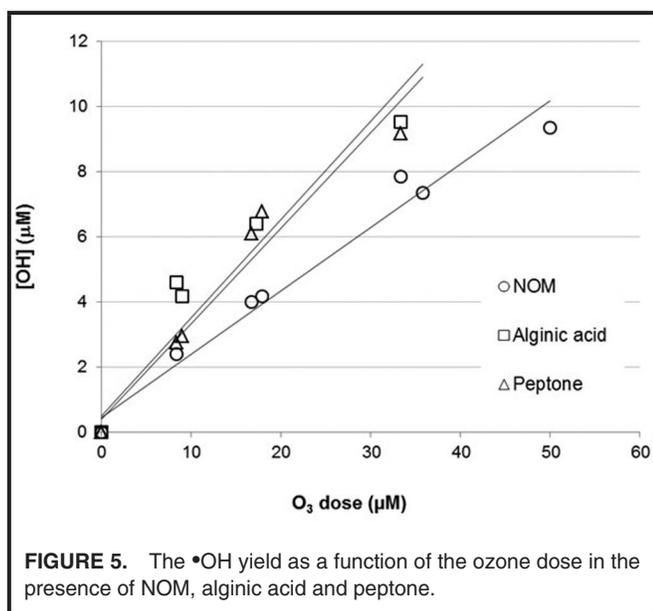
and von Sonntag (2003) for direct reaction of ozone with phenol ($\sim$22%).

The relatively high yield calculated for alginic acid may indicate the formation of •OH through the radical chain reaction. Staehelin and Hoigné (1982) calculated a yield of $\sim$55% for •OH formation in ozonation of pure water, through a radical chain reaction. For peptone, the high yield can probably be attributed to direct reaction of ozone with amino acids, generating high levels of •OH through the intermediate of $O_2^{•-}$ (Buffle and von Gunten 2006). NOM is most similar in structure to the wastewater effluent described by Nöthe et al. (2009), which is rich in humic substances (>61% of TOC) and poor in biopolymers such as carbohydrates and glycoproteins (2.2% of TOC); hence the comparable value of •OH yield.

CONCLUSIONS

Influence of the EfOM Model Compounds on Ozone Decay

- Peptone and NOM were highly reactive toward molecular ozone; therefore at high peptone and NOM concentration ozone decay was fast and controlled by direct reaction with the model compounds. Alginic acid was least reactive toward molecular ozone, thus, ozone decay was relatively slow and controlled by a radical chain reaction.
- Ozone decay through a radical chain reaction was responsible for the relatively high •OH yield calculated for alginic acid; while the high yield found for peptone is probably the result of ozone direct reaction with amino acids.

Influence of the EfOM Model Compounds on CPD Removal

- Cyclophosphamide (CPD) removal occurred mainly through its reaction with •OH radicals and decreased with increasing model compounds concentration.
- The decrease in CPD removal at high model compounds concentration was most pronounced for peptone and least pronounced for alginic acid, due to the differences in their reaction rate constants with •OH (scavenging effect).

Influence of Alkalinity

- Increasing alkalinity decreased CPD removal rate at DOC concentration < 5 mg/L, due to the alkalinity scavenging of •OH. At higher DOC levels ($\geq$5 mg/L), alkalinity had only a minor effect on CPD removal, indicating that the scavenging effect of the EfOM model compounds was the dominant mechanism.

FIGURE 5. The •OH yield as a function of the ozone dose in the presence of NOM, alginic acid and peptone.

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