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Trace level nitrite sensitized photolysis of the antimicrobial agents parachlormetaxylenol and chlorophene in water

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Nitrite (NO_2^-)-sensitized photolysis plays an important role in the attenuation of effluent-derived trace organic contaminants (e.g., anilines, phenolic compounds, etc.) in surface waters. However, the kinetics, mechanisms, and influencing factors of photolysis of many emerging contaminants sensitized by NO_2^- still remain largely unknown. Herein, we report that NO_2^- -sensitized photolysis of the antimicrobial agents parachlormetaxylenol (PCMX) and chlorophene (CP) in aqueous solution under ultraviolet 365 nm (UV_{365}) radiation. A nonlinear increase in photolysis rate constants of PCMX and CP was observed with increasing NO_2^- concentration. Radical quenching studies and kinetic modeling revealed that hydroxyl radical ($\text{HO}\cdot$) and nitrogen dioxide radicals ($\text{NO}_2\cdot$) contributed dominantly to the removal of PCMX and CP. Solid phase extraction (SPE) combined with high resolution-mass spectrometry (HR-MS) analysis identified a series of intermediate products including hydroxylated, nitrated, nitrosated, and dimerized derivatives. Experiments with isotopically labelled nitrite ($^{15}\text{NO}_2^-$) showed that the nitro- and nitroso-substituents of intermediate products were derived from the nitrite nitrogen. Based on the identified products and theoretical computations, the mechanisms and pathways of NO_2^- -sensitized photolysis of PCMX and CP are elucidated. Deoxygenation partially inhibited the formation of 4-chloro-3,5-dimethyl-2-nitrophenol (nitro-PCMX) while the presence of $\text{HO}\cdot$ scavenger such as isopropanol (*i*-PrOH) suppressed the further transformation of nitro-PCMX. The presence of Mississippi River natural organic matter (MRNOM) inhibited the removal of PCMX and CP, likely due to light screening and radical quenching. However, appreciable degradation of PCMX and CP was still observed in wastewater and wetland water matrices. Results of this study shed some light on the transformation and fate of PCMX and CP in NO_2^- -rich wastewater effluents or effluent-impacted surface waters under solar radiation.

1. Introduction

NO_2^- is a well-known photosensitizer which has been implicated in the atmospheric and aqueous photochemistry (Minero et al., 2007). Under artificial or solar radiation, NO_2^- is capable of generating a series of reactive species, including hydroxyl radical ($\text{HO}\cdot$) and reactive nitrogen species (RNS) such as nitric oxide ($\text{NO}\cdot$) and nitrogen dioxide radicals ($\text{NO}_2\cdot$) (Reactions (1)-(3)) (Fischer and Warneck, 1996; Mack and Bolton, 1999).

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These species play critical roles in the transformation and fate of organic contaminants in the environment (Pang et al., 2019; Scholes et al., 2019; Zhou et al., 2020). The concentrations of NO_2^- in surface waters are generally low (e.g., 1 to 3 orders of magnitude lower than that of NO_3^-); however, NO_2^- has a higher absorbance at ultraviolet A (UVA) range which makes it a potentially competitive photosensitizer (Vione et al., 2002; 2003). While the photosensitization of NO_2^- has been less studied in aqueous photochemistry, there is evidence that the quantum yield of $\text{HO}\cdot$ by photolysis of NO_2^- upon UV-visible radiation is comparative to that of NO_3^- (Xu et al., 2019). Therefore, the photosensitization of NO_2^- and its role in the transformation of environmental contaminants have been reevaluated recently (Pang et al., 2019; Scholes et al., 2019; Zhou et al., 2020).

The occurrence of NO_2^- in the environment is mainly derived from the reduction of NO_3^- and, to a less extent, resulted from the degradation of dissolved organic matter (Kieber et al., 1999). A typical concentration of NO_2^- in surface waters is ca. 2×10^{-6} M; however, higher concentrations are likely encountered in municipal wastewater treatment plant (WWTP) effluent due to the nitrification/denitrification processes of microbes (Kroupova et al., 2018). Therefore, when the effluent is released into the aquatic environment and exposed to solar radiation, NO_2^- is expected to influence the fate of effluent-derived contaminants as well as their ecotoxicological risks through photosensitization (Scholes et al., 2019; Zhou et al., 2020). For example, Scholes et al. (2019) proposed that NO_2^- -sensitized photolysis served as an important process responsible for the attenuation of sulfamethoxazole (SMX) in WWTP effluent. The generation of $\text{HO}\cdot$ and RNS during NO_2^- photolysis induce the hydroxylation, nitration, and nitrosation reactions of organic compounds in aqueous solution (Shankar et al., 2007; 2008; Nelieu et al., 2008). In particular, the nitration and nitrosation are undesirable due to the formation of mutagenic and carcinogenic nitro- and nitroso-derivatives (Kovacic and Somanathan, 2014). In addition, the photosensitizing efficiency of NO_2^- is closely related with the molecular structures of contaminants due to the electrophilic properties of $\text{HO}\cdot$ and RNS (Zhou et al., 2020). In general, organic contaminants containing electron-rich moieties, such as anilino and phenolic groups, are susceptible to $\text{HO}\cdot$ and RNS attack (Pang et al., 2019; Scholes et al., 2019). However, except for a few compounds (e.g., SMX), the efficiencies and mechanisms of photolysis of many contaminants sensitized by NO_2^- have not been reported yet.

Parachlorometaxylenol (PCMX) and chlorophene (CP) are two antimicrobial agents widely applied in personal care products including hand sanitizer, soap, and shower gel. For example, hand sanitizers can contain PCMX with concentrations up to 0.18 - 0.22% (W/W). After use, the antimicrobial agents enter municipal sewage mainly through hand washing and bathing (Juksu et al., 2019). The removal of antimicrobial agents by conventional municipal wastewater treatment systems is expected to be limited due to their bactericidal nature (Juksu et al., 2019). Consequently, high concentrations of antimicrobial agents have been detected in WWTP effluent (e.g., the concentration of PCMX reached 1.46 - 5.59 $\mu\text{g L}^{-1}$ in wastewater) (Li et al., 2020). The WWTP effluent, serving as a "hotpot" of pharmaceuticals and personal care products (PPCPs), is an important route through which antimicrobial agents are released into the environment (Michael et al., 2013). The occurrence of antimicrobial agents in the environment has been implicated in a range of endocrinological abnormalities in aquatic species (Sreevidya et al., 2018). These compounds can also be genotoxic and neurotoxic as demonstrated by PCMX and methylisothiazolinone (MIT) (Capkin et al., 2017). Abiotic transformation, such as NO_2^- -sensitized photolysis, is expected to be an important attenuation process affecting the fate of effluent-derived micropollutants such as antimicrobial agents. However, the kinetics, mechanisms, and influencing factors of photolysis of PCMX and CP sensitized by NO_2^- are unknown, which limit the accurate assessment of their environmental fate and risks.

In this contribution, we attempted to investigate the efficiencies and mechanisms of NO_2^- photosensitization in the removal of PCMX and CP in aqueous solution. Reactive species responsible for the degradation of PCMX and CP were discerned by radical quenching studies. The second-order rate constants between the antimicrobial agents and $\text{HO}\cdot/\text{NO}_2\cdot$ were determined either by competition kinetic method or by kinetic modeling. The transformation products of PCMX and CP were identified by high resolution-mass spectrometry (HR-MS) and the conversion of ni-

trite nitrogen was traced by ^{15}N isotope-labeling experiments. In particular, the toxic nitrated byproduct (i.e., nitro-PCMX) was quantified under different reaction conditions by high performance liquid chromatography (HPLC) with an authentic standard. Based on the identified products and theoretical computations, the mechanisms and pathways of NO_2^- -sensitized photolysis of PCMX and CP were elucidated. The effects of river water natural organic matter (NOM) and real waters on the removal of PCMX and CP were also evaluated. Results of this study may shed some light on the transformation and fate of PCMX and CP in NO_2^- -rich wastewater effluents or effluent-impacted surface waters under solar radiation.

2. Materials and methods

2.1. Materials

Chemicals, suppliers, and purities are provided in Text S1 of Supporting Information (SI). All other chemicals were of reagent grade or higher purity. Milli-Q water (18 M Ω cm) was prepared from a Stakpure OmniaTap water purification system (Peculiar Instrument Technology, UK). All the stock solutions were prepared by dissolving chemicals into Milli-Q water and used within one week. Oasis hydrophilic-lipophilic balance (HLB) cartridges (6 cc/200 mg, WAT106202) were purchased from Waters Corporation (Milford, MA, USA).

2.2. Water samples

Two surface waters (i.e., a wetland water and a wastewater) were used to investigate the effects of water matrices. The wetland water was sampled from a shallow macrophyte-dominated wetland in the Xiamafang Park near Nanjing Agricultural University. The wastewater was sampled from a local municipal wastewater treatment plant (Nanjing city, China). Both water samples were filtered through 0.22 μm pore size cellulose acetate membranes prior to use. Characteristics of water samples including TOC, Abs₃₆₅, alkalinity, nitrate, nitrite, and pH are provided in Table S1 of SI.

2.3. Instrumentation

Photolysis experiments were conducted in a multichannel collimated photoreactor (PCX50B Discover, Beijing, China) equipped with nine UV-LED lamps (5W) emitting predominantly 365 nm monochromatic light (UV₃₆₅). The LED lamps were mounted on a rotating dish to ensure that each reactor received uniform light radiation. The photoreactor adopted a bottom-up radiation mode. Incident photon irradiance was determined to be 1.27×10^{-7} Einstein $\text{cm}^{-2} \text{s}^{-1}$ by *para*-nitroanisole/pyridine (PNA/pyr) actinometry (Dulin and Mill, 1982). An integrated air-cooling system was equipped to maintain the temperature at 25 $^{\circ}\text{C}$. A schematic diagram of the photoreactor is shown in Fig. S1 (SI).

The analytes were quantified by a Hitachi L-2000 high performance liquid chromatograph equipped with an L-2200 autosampler, an L-2130 binary pump, and an L-2455 diode array detector (DAD). Solid phase extractions (SPE) were conducted using a Supelco vacuum manifold (Sigma-Aldrich) with HLB cartridges. UV-visible absorption spectra were recorded by a Varian Cary 50 UV-Vis spectrophotometer (Varian, CA, USA) with 1 cm pathlength quartz cuvettes. Solution pH was measured by a PHS-3CW microprocessor pH/mV meter (Bante instruments, Shanghai, China) using an E-201-C model composite glass electrode. NO_2^- and NO_3^- ions were analyzed by an IC 6000 ion chromatograph (Wanyee, Anhui, China) equipped with a Shodex IC SI-52 4E column (250 mm length $\times$ 5 mm, i.d.), a Shodex IC SI-92G pre-column, and a conductivity detector.

Concentrations of PCMX, CP, nitro-PCMX, *p*CBA, and PNA were quantified by the L-2000 HPLC. Analytical separation was performed by an Agilent Zorbax Eclipse Plus C18 column (5.0 μ m particle size, 250 mm length $\times$ 4.6 mm, i.d.). Mobile phase was a combination of eluent A (methanol with 0.1% formic acid) and eluent B (H₂O with 0.1% formic acid) at a flow rate of 1.0 mL min⁻¹. Detailed HPLC parameters can be found in Table S2 of SI. Quantification of analytes was based on multipoint standard calibration curves.

The intermediate products were identified by HPLC (Shimadzu, Kyoto, Japan) coupled with a Triple TOF 5600⁺ mass spectrometer (LC-qTOF-MS/MS, AB Sciex, Boston, USA). Separation was accomplished using a Kinetex LC C18 column (2.6 μ m, 100Å, 100 mm $\times$ 2.1 mm, i.d.). Elution was performed at a flow rate of 0.3 mL min⁻¹ with 50% methanol (eluent A) and 50% Milli-Q water (eluent B), employing an isocratic gradient. Sample injection volume was 2 μ L. Mass analyses were conducted in negative mode using an electrospray ionization (ESI) source. Instrumental parameters were as follows: ionspray voltage floating of 5500 V; source temperature of 550°C; gas I and gas II of 65 psi; curtain gas of 35 psi. Mass spectrometric data were collected in TOF MS full scan mode over a mass range of 50 - 800 *m/z*, and MS/MS scan mode (scan range of 50 - 800 *m/z*) was triggered by information-dependent acquisition (IDA) workflow at the same time. The MS/MS analyses were conducted in negative ion mode. Declustering potential, collision energy, and collision energy spread were -80 V, -30eV, and 10 eV, respectively.

2.4. Photolysis experiments

The photolysis experiments were conducted in a series of cylindrical flat-bottomed quartz tubes (31 mm i.d., 78 mm height, 59 mL volume). 50 mL solutions containing 20 μ M PCMX/CP and certain amounts of NO₂⁻ (2 to 100 μ M) were transferred into the tubes. The solutions were buffered by 1 mM phosphate (pH 7.0) to represent circumneutral pH.

The photoreactor was turned on for 15 min for stabilization, and the tubes were then put into the photoreactor for radiation. Dark controls (wrapped with aluminum foil) and direct photolysis (in the absence of NO₂⁻) were run concurrently. In some experiments, excess scavengers (i.e., *i*-PrOH and TBA) were spiked into solutions prior to photolysis to discern the contribution of HO• and RNS. The effects of O₂ were explored by purging purified N₂ into solutions over radiation to achieve anaerobic conditions. Different concentrations of MRNOM were added into solutions to evaluate the effects of river water NOM. The photolysis experiments were also carried out in wetland water and wastewater amended with 20 μ M PCMX/CP and 100 μ M NO₂⁻. Aliquots (1 mL) were withdrawn at predetermined time intervals and transferred to 1.5 mL amber HPLC vials. Samples were refrigerated at 4 °C and analyzed by HPLC within 24 h. All experiments were carried out in duplicates to assure accurate data acquisition and error bars in figures represent standard deviations.

2.5. Isolation and identification of intermediate products

To identify intermediate products, two reaction solutions (50 mL, pH 7.0 buffer) containing 20 μ M PCMX/CP and 100 μ M NO₂⁻ were freshly prepared and then put into the photoreactor for radiation. Specifically, a mixture of 50% ¹⁴NO₂⁻ + 50% ¹⁵NO₂⁻ was also used as photosensitizer to trace whether the nitroso- and nitro- substituents of intermediate products were derived from nitrite nitrogen (Kolkman et al., 2015). After 120 min of radiation, the photolytic solutions were withdrawn and then subjected to SPE enrichment using HLB cartridges. Detailed procedures of SPE can be found in our previous studies (Ji et al., 2018). The SPE-concentrated

samples were analyzed by the high resolution-mass spectrometry (LC-qTOF-MS/MS) for identification of intermediate products.

2.6. Determination of second-order rate constants between HO• and PCMX/CP

Second-order rate constants for reactions of HO• with PCMX/CP were determined by competition kinetic method in a UV/H₂O₂ system (Baeza and Knappe, 2011). HO• is known as the sole free radical generated in the UV/H₂O₂ system (Miklos et al., 2018). Detailed experimental procedures can be found in Text S2 of SI. The second-order rate constants between HO• and PCMX/CP are calculated by the following equation (Fig. S2, SI):

$$k_{\text{HO}\cdot, \text{AA}} = \frac{\ln([AA]_t/[AA]_0)}{\ln([p\text{CBA}]_t/[p\text{CBA}]_0)} k_{\text{HO}\cdot, p\text{CBA}} \quad (1)$$

where [AA]_t and [AA]₀ are the concentrations of the antimicrobial agents at time *t* and zero, respectively; [pCBA]_t and [pCBA]₀ are the concentrations of *para*-chlorobenzoic acid at time *t* and zero, respectively; *k*_{HO•,AA} and *k*_{HO•,pCBA} are the second-order rate constants for HO• reactions with the antimicrobial agents and *p*CBA, respectively.

2.7. Computational study

In the present study, frontier electron densities (FEDs) of high-est occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) were computed using a Gaussian 09 software package at B3LYP/6-311+G* level. As reported, the values of 2FED²_{HOMO} can be used to predict the reactive sites susceptible for electron transfer reaction, while the values of FED²_{HOMO}+FED²_{LUMO} can be used to predict the sites susceptible to electrophilic radical addition (Qu et al., 2015; Zeng et al., 2016).

3. Results and discussion

3.1. Reaction kinetics

Direct photolysis of PCMX and CP was not observed under UV₃₆₅ radiation (data not shown), consistent with the fact that the antimicrobial agents have no absorbance at 365 nm (Fig. S3, SI). Under circumneutral condition (pH 7.0), PCMX and CP were also found to be stable in the dark in the presence of NO₂⁻ (dark control, data not shown). This is reasonable because NO₂⁻-induced dark reactions generally proceed through electrophilic aromatic substitutions (S_EAr), which require a strongly acidic condition to generate nitrating/nitrosating agents such as nitrosonium ion (NO⁺) (Patnaik and Khoury, 2004; Kroflič et al., 2018). Exposure of PCMX and CP solutions to UV₃₆₅ radiation in the presence of trace level NO₂⁻ (2 - 100 μ M) resulted in rapid consumption of these antimicrobial agents (Fig. S4, SI), suggesting that reactive species resulted from NO₂⁻ photolysis were responsible for their removal. The degradation of PCMX obeyed pseudo-first-order reaction kinetics. The observed pseudo-first-order rate constant (*k*_{obs}), which was obtained by plotting -ln([PCMX]_t/[PCMX]₀) as a function of irradiation time *t*, increased by a factor of 18.6 (i.e., 0.15 - 2.79 $\times$ 10⁻⁴ s⁻¹ with half-life times 12.83 - 0.69 h) as the NO₂⁻ concentration was elevated from 2 to 100 μ M (Fig. 1).

A similar increment was also observed in NO₂⁻-sensitized photolysis of CP, with *k*_{obs} value being increased by 20.9-fold (i.e., 0.13 - 2.61 $\times$ 10⁻⁴ s⁻¹ with half-life times 15.4 - 0.74 h) (Fig. 1). Intriguingly, the observed *k*_{obs} value did not increased proportionally with the concentration of NO₂⁻. The lack of a linear relationship between *k*_{obs} and NO₂⁻ concentration is possibly due to the scavenging of HO• by excess NO₂⁻ (Shankar et al., 2007; Chen et al., 2015). Because NO₂⁻ reacts with HO• at a diffusion-controlled rate

Fig. 1. The observed pseudo-first-order rate constant (k_{obs}) of photolysis of (●) PCMX and (●) CP sensitized by NO_2^- with varying concentrations. Experimental conditions: 20 μM PCMX or CP, 0 - 100 μM NO_2^- , 1 mM phosphate buffer (pH 7.0). Error bars represent the standard deviations ($n = 2$).

($1.0 \times 10^{10} M^{-1}s^{-1}$), excess NO_2^- can serve as a dominant sink of $HO\bullet$ and, therefore, reduce the steady-state concentration of $HO\bullet$ (Reaction (3)) (Vione et al., 2001; García Einschlag et al., 2009).

Irradiation of aqueous NO_2^- solution results in the generation of NO_3^- as an important inorganic product (De Laurentiis et al., 2015). Preliminary experiment revealed that upon UV_{365} radiation, 12.2% of NO_2^- (101.8 μM) was stoichiometrically converted to NO_3^- (12.4 μM) after 2 h of photolysis (Fig. S5, SI). In the presence of PCMX, the yield of NO_3^- was increased up to 25.8% corresponding to 25.8 μM . The increased formation of NO_3^- in the presence of organic compound during NO_2^- photolysis has previously been reported and was ascribed to the enhanced generation of $O_2^{\bullet-}$ and $NO\bullet$ (De Laurentiis et al., 2015).

3.2. Reactive species

The involvement of $HO\bullet$ and RNS in the photolysis of PCMX and CP was probed by quenching studies using two scavengers, namely *i*-PrOH and TBA. While both alcohols react with $HO\bullet$ at high second-order rate constants (i.e., 1.9×10^9 and $6.8 \times 10^8 M^{-1}s^{-1}$, respectively), they are known to be inert with RNS (Zhou et al., 2020) (see below). However, the quenching of $HO\bullet$ by scavengers can result in a decline of $NO_2^{\bullet}$ because it is mainly derived from $HO\bullet$ reaction with NO_2^- in UV_{365}/NO_2^- system (Scholes et al., 2019).

The presence of 10 mM *i*-PrOH decreased the k_{obs} values of PCMX and CP by 82.7% and 84.7%, respectively (Fig. S6, SI). In the case of 10 mM TBA as scavenger, the k_{obs} values reduced by 69.1% and 66.7%, respectively. The greater inhibitory effect of *i*-PrOH relative to TBA is in good agreement with their reactivities with $HO\bullet$ (Scholes et al., 2019). Kinetic calculations suggested that the addition of 10 mM *i*-PrOH/TBA quenched 96%/84% of $HO\bullet$, which in turn led to an 80%/60% decrease in $NO_2^{\bullet}$ (see Table S3 and detailed calculations in Text S3, SI). Therefore, the above results highlight the important roles of $HO\bullet$ and $NO_2^{\bullet}$ in NO_2^- -sensitized photolysis of PCMX and CP, which are consistent with various hydroxylated and nitrated products identified by HR-MS (*vide infra*). The second-order rate constants for reactions of $HO\bullet$ with PCMX/CP were determined to be 2.74×10^9 and $6.94 \times 10^9 M^{-1}s^{-1}$, respectively, by competition kinetic method (Text S2 and Fig. S2, SI). These values approach diffusion-controlled limit in water (i.e.,

$7.4 \times 10^9 M^{-1}s^{-1}$), suggesting the high reactivities of antimicrobial agents with $HO\bullet$. The observation that the removal of antimicrobial agents was not completely inhibited (e.g., for PCMX, the k_{obs} values were $3.18 - 0.55 \times 10^{-4} s^{-1}$ and the removal percentages were 92.64 - 32.49%) in the presence of excess scavengers implies that other reactive species (e.g., $NO\bullet$, N_2O_3 , $ONOO^-/ONOOH$, etc.) also contributed to the degradation. However, these species are not expected to be important in the removal of antimicrobial agents due to their weaker redox potential (Xu et al., 2019).

Considering that $HO\bullet$ and $NO_2^{\bullet}$ are dominant reactive species in NO_2^- -sensitized photolysis of antimicrobial agents, the transformation rate contributed by $NO_2^{\bullet}$ can be evaluated by the following equation (Scholes et al., 2019):

$$k_{NO_2^{\bullet}} = k_{obs} - k_{HO\bullet} = k_{obs} - k_{HO\bullet,AA}[HO\bullet]_{ss} \quad (2)$$

where $k_{HO\bullet}$ is the transformation rate caused by $HO\bullet$ reaction; $k_{HO\bullet,AA}$ is the second-order rate constant between $HO\bullet$ and the antimicrobial agent; and $[HO\bullet]_{ss}$ is the steady-state concentration of $HO\bullet$. The $k_{NO_2^{\bullet}}$ value can be calculated by the following equation:

$$k_{NO_2^{\bullet}} = k_{NO_2^{\bullet},AA}[NO_2^{\bullet}]_{ss} \quad (3)$$

where $k_{NO_2^{\bullet},AA}$ is the second-order rate constant between $NO_2^{\bullet}$ and the antimicrobial agent; and $[NO_2^{\bullet}]_{ss}$ is the steady-state concentration of $NO_2^{\bullet}$. It is assumed that in UV_{365}/NO_2^- system, $NO_2^{\bullet}$ is generated predominantly by $HO\bullet$ reaction with NO_2^- , and the dominant fate of $NO_2^{\bullet}$ is dimerization followed by hydrolysis (Reactions (4) and (5)) (Minero et al., 2007).

Therefore, according to a steady-state approximation method, $[HO\bullet]_{ss}$ and $[NO_2^{\bullet}]_{ss}$ can be calculated by the following equations (see Text S4 of SI for detailed derivation):

$$[HO\bullet]_{ss} = \frac{2.3E_p^0 \varepsilon_{NO_2^-} \Phi_{NO_2^- \rightarrow HO\bullet} [NO_2^-]}{k_{HO\bullet,NO_2^-} [NO_2^-] + k_{HO\bullet,AA} [AA]} \quad (4)$$

$$[NO_2^{\bullet}]_{ss} = \sqrt{\frac{k_{HO\bullet,NO_2^-} (k_{-1} + k_2) [HO\bullet]_{ss} [NO_2^-]}{2k_1 k_2}} \quad (5)$$

where E_p^0 is the incident photon irradiance; $\varepsilon_{NO_2^-}$ is the molar absorption coefficient of NO_2^- at 365 nm; and $\Phi_{NO_2^- \rightarrow HO\bullet}$ is the quantum yield of $HO\bullet$ by NO_2^- photolysis; k_1 , k_{-1} , and k_2 are the rate constants of forward, reverse, and hydrolysis reactions of N_2O_4 , respectively. We performed a linear regression of $k_{NO_2^{\bullet}}$ versus calculated $[NO_2^{\bullet}]_{ss}$ and obtained $k_{NO_2^{\bullet},AA}$ values of 2.3×10^4 and $1.1 \times 10^4 M^{-1}s^{-1}$ for PCMX and CP, respectively (Fig. 2). The $k_{NO_2^{\bullet},AA}$ value of PCMX is higher than those of other phenolic compounds reported in literature, e.g., $3.2 \times 10^3 M^{-1}s^{-1}$ for phenol and $1.1 \times 10^4 M^{-1}s^{-1}$ for 4-chlorophenol (Maddigapu et al., 2010), possibly due to the electron donating effect of methyl groups. Given that $[NO_2^{\bullet}]_{ss}$ in surface water is typically $10^{-11} - 10^{-9} M$ (Minero et al., 2007), the half-life times of PCMX and CP are deduced to be 8.4 - 836 and 17.5 - 1750 h, respectively. This result implies that $NO_2^{\bullet}$ may play an important role in the attenuation of PCMX and CP in sunlit surface waters containing high concentrations of NO_2^- . Interestingly, the $k_{NO_2^{\bullet},AA}$ value of CP is exactly the same as that of 4-chlorophenol, implying that the benzyl moiety is inert with respect to $NO_2^{\bullet}$ reaction. This result corroborates the fact that $NO_2^{\bullet}$ is not as strong an electrophilic radical ($E^0 (NO_2^{\bullet}/NO_2^-) = 1.04 V$) and its reaction is regioselective (Vione et al., 2001).

Fig. 2. Linear regression of $k_{\text{NO}_2\bullet}$ versus calculated $[\text{NO}_2\bullet]_{\text{ss}}$ to obtain $k_{\text{NO}_2\bullet\text{AA}}$ values for (●) PCMX ($2.3 \times 10^4 \text{ M}^{-1}\text{s}^{-1}$) and (●) CP ($1.1 \times 10^4 \text{ M}^{-1}\text{s}^{-1}$). The value of $k_{\text{NO}_2\bullet}$ was calculated by Eq. (2), and the value of $[\text{NO}_2\bullet]_{\text{ss}}$ was calculated by Eqs. (4) and (5). Experimental conditions: 20 μM PCMX or CP, 0 – 100 μM NO_2^- , 1 mM phosphate buffer (pH 7.0).

3.3. Intermediate products and transformation pathways

The intermediate products generated in NO_2^- -sensitized photolysis of PCMX and CP were concentrated by SPE and then identified by HR-MS. The assignments of intermediate products were based on the following criteria: (i) the detected exact masses with error less than 5 ppm of the theoretical exact masses, (ii) the fragmentation patterns of molecular ions, (iii) the characteristic isotope distribution endowed by the chlorine atom, and (iv) comparison to the exact masses and retention times of authentic standards (commercially available). In particular, isotopically labeled nitrite ($^{15}\text{NO}_2^-$) was used as a nitrogen source to trace if the nitroso- and nitro-substituents of intermediate products were derived from NO_2^- (Kolkman et al., 2015). Overall, a series of products including hydroxylated, nitrated, nitrosated, and dimerized derivatives were temporarily identified (Table S4 and S5, SI), providing further evidence that $\text{HO}\bullet$, $\text{NO}_2\bullet$, and $\text{NO}\bullet$ were involved in the NO_2^- -sensitized photolysis of PCMX and CP (Ge et al., 2019).

As expected, $\text{HO}\bullet$ produced by photolysis of NO_2^- upon UV_{365} radiation can lead to the hydroxylation of PCMX. Interestingly, two isomers of monohydroxylated PCMX (**P-1**) were identified by HR-MS, implying that hydroxylation likely occurred at different positions of PCMX molecule (i.e., the aromatic ring or the methyl side chain). Further support in favor of hydroxylation at different positions was obtained by examining the fragmentation patterns provided by HR-MS (Fig. S7, SI). The lack of fragment with m/z 79.0595 derived from consecutive loss of 28 Da ($-\text{CO}$) possibly suggested that the hydroxylation occurred at the methyl group. In addition, computational studies revealed the high $\text{FED}^2_{\text{HOMO}} + \text{FED}^2_{\text{LOMO}}$ values of the unsubstituted positions (C2 and C4), indicating that the *ortho*-sites with respect to the phenolic hydroxyl group are susceptible to the electrophilic substitution of $\text{HO}\bullet$ (Table S6, SI). While the $\text{FED}^2_{\text{HOMO}} + \text{FED}^2_{\text{LOMO}}$ values of the methyl-substituted positions (C1 and C5) are also high, these positions are unlikely to be hydroxylated due to the steric hindrance of the bulky methyl groups that will reduce the accessibility of $\text{HO}\bullet$ (Chen et al., 2015). The addition of $\text{HO}\bullet$ at aromatic ring is known to generate a hydroxycyclohexadienyl (HCHD) radical, which undergoes further O_2

addition and hydroxyl peroxide radical ($\text{HO}_2\bullet$) elimination, giving rise to a hydroxylated product (Scheme 1 of Fig. 3) (Mvula et al., 2001). In the case of side chain substitution with $\text{HO}\bullet$ attacking one of the methyl groups, the reaction is likely initiated by H-abstraction, resulting in the formation of a carbon-centered benzyl radical that combines with another $\text{HO}\bullet$ to generate a carbinol product ($\text{Ph-CH}_2\text{-OH}$) (Scheme 2 of Fig. 3) (Pignatello et al., 2006). It has been reported that the H-abstraction of alkyl side chain by $\text{HO}\bullet$ attack generally occurs at a rate constant much slower than that of ring addition (Gligorovski et al., 2015). In addition, the methyl positions (C7 and C8) possess much lower $\text{FED}^2_{\text{HOMO}} + \text{FED}^2_{\text{LOMO}}$ values (0.0026 and 0.0021), suggesting that the ring hydroxylation is more favorable than the methyl group hydroxylation. The initial hydroxylation will increase the electron density of the aromatic ring, thus favoring further hydroxylation under the attack of $\text{HO}\bullet$. Therefore, the consecutive attack of $\text{HO}\bullet$ can lead to the formation of dihydroxylated (**P-2**) and trihydroxylated products (**P-3**). It should be emphasized that unequivocal structural assignment of the hydroxylated (mono-, di-, and tri-) products is impossible at the current stage due to the lack of reference standards, and additional spectroscopic analyses such as nuclear magnetic resonance (NMR) are necessary to ascertain the exact hydroxylated positions. Polyhydroxylated products are rich in electron density and thus are susceptible to ring-opening, giving rise to various carboxylic acids and aldehydes (Yang et al., 2020). It should further be noted that SPE used to concentrate photoproducts may result in the loss of highly polar compounds, in particular those low-molecular-weight compounds such as aromatic ring cleaving products. Therefore, additional experiments with elevated contaminant concentrations (which do not require a preconcentration step) are essential to determine whether any additional products are present.

$\text{NO}_2\bullet$ generated from $\text{UV}_{365}/\text{NO}_2^-$ is capable of nitrating parent compound, as evidenced by the detection of 4-chloro-3,5-dimethyl-2-nitrophenol (nitro-PCMX) (**P-4**, exact mass m/z 200.0122/202.0099 with an isotopic abundance ratio of 3:1 endowed by the chlorine atom, Δppm 0.9). This nitro-derivative was unequivocally confirmed by ^{15}N isotope-labeling experiments (Fig. 4) as well as by comparison with an authentic standard (CAS 54852-90-0). When 50% $^{14}\text{NO}_2^- + 50\%$ $^{15}\text{NO}_2^-$ was used as nitrogen source, HR-MS spectrum revealed the formation of $^{15}\text{NO}_2$ -PCMX (exact mass m/z 201.0096/203.0063; Δppm 0.9) with molecular weight 1 Da greater than that of $^{14}\text{NO}_2$ -PCMX, indicating that the nitro group of nitro-PCMX was derived from the NO_2^- nitrogen (Kolkman et al., 2015). NO_2^- -induced photonitration of aryl compounds has been widely reported (Machado and Boule, 1995; Vione et al., 2004;2005; Calza et al., 2012; Shankar et al., 2007;2008; Nelieu et al., 2008; Chen et al., 2015). In general, the nitration of phenolic compounds by $\text{NO}_2\bullet$ is known to proceed through two-stage reactions (Barletta et al., 2000; Barzaghi and Herrmann, 2002). The initial reaction involves H-abstraction between $\text{NO}_2\bullet$ and substituted phenols (Shenghur et al., 2014). In the case of PCMX containing 4-chlorophenol moiety, the reaction is likely initiated by abstraction of phenolic hydrogen, leading to the formation of a phenoxyl radical and HONO , as evidenced by DFT calculations (Bedini et al., 2012; Ji et al., 2019b; Zhao et al., 2020). The phenoxyl radical can be stabilized via delocalization of its unpaired electron to *ortho*- or *para*-positions through resonance. Subsequently, the combination of phenoxyl radical with another $\text{NO}_2\bullet$ generates 4-chloro-2-nitrocyclohexa-2,5-dienone (Clewley et al., 1989). Such dienone is known to be unstable and undergoes rearrangement with the assistance of a H_2O molecule, resulting in the formation of nitro-derivative (Ji et al., 2017; Chen et al., 2020) (Scheme 3 of Fig. 3). Nitro-PCMX may also be generated by coupling reaction of $\text{NO}_2\bullet$ with the $\text{HO}\bullet$ -adduct (i.e., HCHD radical) followed by the elimination of a H_2O molecule (Dzengel et al., 1999; Barzaghi and Herrmann, 2002).

Fig. 3. Detailed mechanisms accounting for the formation of hydroxylated and nitrated byproducts during NO_2^- -sensitized photolysis of PCMX.

● Spectrum from 2.wiff (sample 1) - Sample002, Experiment 1, -TOF MS (50 - 800) from 7.607 to 7.639 min
 ● $\text{C}_8\text{H}_8\text{ClNO}_3\text{-H}$

Fig. 4. Isotopically labelled experiment combined with HR-MS analysis reveals the formation of nitro-PCMX (exact mass m/z 200.0122/202.0099; Δppm 0.9) in NO_2^- -sensitized photolysis of PCMX. Note that, when 50% $^{14}\text{NO}_2^-$ + 50% $^{15}\text{NO}_2^-$ was used as nitrogen source, $^{15}\text{NO}_2^-$ -PCMX (exact mass m/z 201.0096/203.0063; Δppm 0.9) with molecular weight 1 Da greater than that of $^{14}\text{NO}_2^-$ -PCMX was identified, indicating that the nitro group of nitro-PCMX was derived from the NO_2^- nitrogen. The formation of nitro-PCMX was further confirmed by comparison with an authentic standard (CAS, CAS 54852-90-0) via HPLC analysis.

However, the $\text{HO}\cdot$ -adduct readily undergoes ring-opening in the presence of oxygen (Dzengel et al., 1999), which may inhibit the formation of nitrated byproducts. The coupling reaction between $\text{NO}_2\cdot$ and the $\text{HO}\cdot$ -adduct is also expected to be less significant at high concentration of NO_2^- , because the steady-state concentration of $\text{HO}\cdot$ is low due to the scavenging of $\text{NO}_2\cdot$ (Vione et al., 2001; García Einschlag et al., 2009). Nitration can also be initiated by H-abstraction from the methyl group by photochemically produced $\text{HO}\cdot$ or other radicals. Such reaction is known to generate a benzyl radical (Elias et al., 2011). The addition of $\text{NO}_2\cdot$ to the benzyl radical gives rise to a phenylnitromethane ($\text{Ph-CH}_2\text{-NO}_2$), as observed in the nitration of toluene in irradiated nitric solution (Elias et al., 2011). It is noteworthy that no dinitro- or polynitro-derivatives were detected in the present study. This result can be explained by the fact that the first nitro group can reduce the electron density of aromatic ring by resonance and induction and, therefore, decrease the reactivity of the molecule. How-

ever, nitro-PCMX can undergo further hydroxylation via $\text{HO}\cdot$ attack to generate polyhydroxylated intermediates (**P-5**, **P-6**). These hydroxylated-nitrated-derivatives are also likely formed via nitration of (poly)hydroxylated PCMX (**P-1**, **P-2**), as described in Fig. 5.

Isotope-labeling experiment combined with HR-MS analysis also confirmed the generation of nitrosated product of PCMX (**P-7**, exact mass m/z 184.0174/186.0088, Δppm 1.6). Similar to nitration, the nitrosation is likely resulted from the coupling reaction of $\text{NO}\cdot$ with the resonance-stabilized radical or the benzyl radical. The former will lead to a ring-substituted product; while the latter will result in a benzaldehyde oxime by $\text{NO}\cdot$ addition followed with a rearrangement ($\text{Ph-CH}_2\text{-NO} \rightarrow \text{Ph-CH=NOH}$) (Elias et al., 2011). However, nitroso-PCMX is also likely generated by nitrosation with N_2O_3 , an important nitrosating agent that can be formed by coupling of $\text{NO}\cdot$ with $\text{NO}_2\cdot$ in $\text{UV}_{365}/\text{NO}_2^-$ system (Vione et al., 2001). **P-7** would have undergone further transformation during NO_2^- photolysis; however, our HR-MS analysis did not detect any

Fig. 5. Transformation pathways of NO_2^- -sensitized photolysis of PCMX in aqueous solution. Note that, the nitrated and nitrosated products with substitution occurring at the methyl group were not showed in this figure for readability issues.

transformation products of **P-7** (e.g., the dimer or hydroxylation products). While the reason is unclear, one possible explanation is that **P-7** was quickly converted to the nitrated product **P-4** in the presence of dissolved oxygen (Vione et al., 2001).

Interestingly, HR-MS analysis identified a dimer of PCMX (**P-8**, exact mass m/z 293.0584/295.0557/297.0530, Δppm 2.5), which might be originated from coupling reaction between phenoxyl radical and its resonance-stabilized radical (Ji et al., 2019a). The formation of dimer evidences the involvement of phenoxyl radicals as important precursors in $\text{UV}_{365}/\text{NO}_2^-$ system. It should be noted that dimerization consumes a fraction of phenoxyl radicals, which will diminish the nitration of PCMX. The dimer is likely subjected to further hydroxylation and/or nitration, leading to the formation of **P-9**, **P-10**, and **P-11** (Fig. 5).

The transformation pathways of CP photolyzed in the presence of NO_2^- are illustrated in Fig. S8 of SI. Most notably, the photosensitization of NO_2^- resulted in the formation of nitrated CP (**C-4**), as confirmed by isotope-labeling experiments coupled with HR-MS analysis (Table S5 and Fig. S9, SI). The identification of **C-4** suggests that $\text{NO}_2^\bullet$ was also involved in the transformation of CP, which is consistent with the results of radical quenching studies. Due to its electrophilic nature and regioselectivity (Bedini et al., 2012; Ji et al., 2019b), $\text{NO}_2^\bullet$ most likely attacks the 4-chlorophenol moiety of CP rather than the benzyl moiety. This hypothesis is justified by the computational study showing the higher electron density of chlorophenolic ring (Table S7, SI). Another piece of evidence is the kinetic result showing that both CP and 4-chlorophenol react with $\text{NO}_2^\bullet$ at the same rate constant (i.e., $1.1 \times 10^4 \text{ M}^{-1}\text{s}^{-1}$) (Maddigapu et al., 2010). As mentioned previously, the H-abstraction of CP induced by $\text{NO}_2^\bullet$ attack generates a phenoxyl radical that coupled with another $\text{NO}_2^\bullet$ to generate nitro-CP.

Both CP and nitro-CP can undergo further hydroxylation under the attack of $\text{HO}^\bullet$, giving rise to mono- (**C-1** and **C-5**), di- (**C-2** and **C-6**), and tri-hydroxylated derivatives (**C-3** and **C-7**) sequentially. It should be noted that six mono-hydroxylated isomers were detected by extracted ion chromatograms (data not shown), suggesting that the attack of $\text{HO}^\bullet$ could occur at different positions of CP molecule. In order to determine the exact positions where $\text{HO}^\bullet$ substituted, further analytical methods such as NMR are required.

While the nitrosated product of CP was not identified by HR-MS, we cannot rule out the possibility that nitrosation occurred during NO_2^- -sensitized photolysis of CP. The most reasonable explanation of this observation, as discussed above, is the quick transformation of nitroso-CP in the presence of dissolved oxygen (Vione et al., 2001). In addition, no dimeric products were detected, possibly due to the steric hindrance of the bulky benzyl moiety.

3.4. Tracing nitro-PCMX

As nitro-derivatives can be mutagenic and carcinogenic (Kovacic and Somanathan, 2014), their formation in $\text{UV}_{365}/\text{NO}_2^-$ system should be scrutinized. In the present study, the formation of nitro-PCMX under different conditions was quantified because its authentic standard is commercially available. Irradiation of an aqueous solution containing $20 \mu\text{M}$ PCMX and $100 \mu\text{M}$ NO_2^- resulted in the quick formation of nitro-PCMX with its concentration reaching maximum at 2.5 h ($3.27 \mu\text{M}$, 16.4% yield) (Fig. 6). This product decreased appreciably thereafter and disappeared completely after 4 h.

The evolution of nitro-PCMX was highly dependent on reaction conditions. Under anaerobic condition (N_2 purging), the formation rate of nitro-PCMX was lower than that observed in control ex-

Fig. 6. Evolution profiles of nitro-PCMX during NO_2^- -sensitized photolysis of PCMX under different reaction conditions: (●) control, (■) N_2 -purging to remove dissolved oxygen, and (▲) in the presence of 10 mM *i*-PrOH as $\text{HO}\cdot$ scavenger. Experimental conditions: 20 μM PCMX, 100 μM NO_2^- , 1 mM phosphate buffer (pH 7.0).

periment (Fig. 6). This finding can be rationalized by two facts. (i) Deoxygenation blocked some pathways that otherwise led to the formation of $\text{NO}_2\cdot$. It has been reported that O_2 is conducive to the radical chain propagations of $\text{NO}\cdot$, which in turn favors the formation of $\text{NO}_2\cdot$ (Reactions (4)-(10)). For example, O_2 is necessary for the formation of $\text{O}_2^{\cdot-}/\text{HO}_2\cdot$ that combine with $\text{NO}\cdot$ to produce $\text{ONOO}^-/\text{ONOOH}$. The decomposition of $\text{ONOO}^-/\text{ONOOH}$ can lead to the formation of $\text{HO}\cdot$ and $\text{NO}_2\cdot$ (Reactions (4)-(7)) (Pang et al. 2019). (ii) N_2 purging inhibited direct oxidation of nitroso-PCMX to nitro-PCMX. This explanation is supported by previous studies showing that the nitrophenols observed in UV/NO_2^- system were at least partly derived from direct oxidation of nitrosophenols with O_2 (Vione et al., 2001). However, the decay pro-

file of nitro-PCMX under anaerobic condition was similar to that observed in control experiment. This finding may be ascribed to the fact that the further transformation of nitro-PCMX was mediated dominantly by $\text{HO}\cdot$ (see Fig. 5), whose generation was less affected by dissolved oxygen.

The addition of *i*-PrOH (10 mM) did not affect the formation rate of nitro-PCMX significantly (Fig. 6), consistent with the low reactivities between *i*-PrOH and RNS (e.g., $\text{NO}_2\cdot$ and $\text{NO}\cdot$) (Zhou et al., 2020). However, the presence of *i*-PrOH slowed the decay rate of nitro-PCMX, suggesting that $\text{HO}\cdot$ was the leading oxidant for further transformation of nitro-PCMX (*vide supra*). This is reasonable considering that nitrated byproducts are more recalcitrant than their parent compound and are resistant to oxidation by RNS (Chen et al., 2020). The above results led us to speculate that other $\text{HO}\cdot$ scavengers, such as NOM, may also retard the decay of nitro-derivatives in $\text{UV}_{365}/\text{NO}_2^-$ system, which warrants further investigations.

3.5. Effects of water matrices

NOM that is ubiquitously present in natural waters may have significant impacts on NO_2^- -sensitized photolysis of organic contaminants (Minero et al., 2007). Fig. 7 shows that the presence of MRNOM, as a representative of aquatic NOM, inhibited the degradation of PCMX and CP in $\text{UV}_{365}/\text{NO}_2^-$ system, and the inhibitory

Fig. 7. The effects of MRNOM, as a representative of river water NOM, on NO_2^- -sensitized photolysis of (a) PCMX and (b) CP. Experimental conditions: 20 μM PCMX or CP, 100 μM NO_2^- , 1 mM phosphate buffer (pH 7.0). Error bars represent the standard deviations ($n = 2$).

effect was exacerbated as MRNOM concentration increased. For example, when MRNOM concentration was elevated from 0 to 5 mgL⁻¹, the observed k_{obs} value of PCMX decreased from 0.0109 ± 0.0002 to 0.0037 ± 0.0003 min⁻¹. It should be noted that no photosensitizing effects of MRNOM (via triplet excited state (³NOM*) or ROS) were observed (data not shown). The inhibitory effect of MRNOM can be rationalized by following reasons. First, the chromophores of NOM, such as aryl ketones, are capable of absorbing photons at 365 nm wavelength (McNeill and Canonica, 2016), thus limiting the generation of reactive species by NO₂⁻ photolysis. On the other hand, the electron-rich moieties (ERMs) of MRNOM, such as phenolic groups, have a potential to scavenge reactive radicals (Aeschbacher et al., 2010). These results imply that when wastewater is released into rivers, the photolysis of contaminants sensitized by NO₂⁻ can be reduced, mostly likely due to the light screening and radical quenching capacity of river water NOM.

NO₂⁻-sensitized photolysis of PCMX and CP was also found to be appreciable in wastewater and wetland water matrices (Fig. S10, SI), in agreement with a previous study (Scholes et al., 2019). This result highlights the potential importance of NO₂⁻ photosensitization to the attenuation of antimicrobial agents in real water matrices upon solar radiation. It is interesting to note that, while wastewater contained greater level of NOM (10.5 mg/L TOC) than wetland water (5.0 mg/L TOC), higher degradation rate constants of PCMX and CP were observed in the former matrix. One possible reason is the very high alkalinity of wetland water (Table S1 of SI), which may affect the photochemistry of NO₂⁻ (Chiron et al., 2009). It is well-known that bicarbonate and carbonate, the major contributors of alkalinity, are important HO• scavengers in fresh waters, and the resulting carbonate radical (CO₃•⁻) can significantly scavenge NO₂• (Reactions (11)–(13)) (Chiron et al., 2009).

4. Conclusions and environmental implications

Exposure of aqueous PCMX and CP solutions to UV₃₆₅ radiation in the presence of trace level NO₂⁻ led to the rapid decay of these antimicrobial agents. The NO₂⁻-sensitized photolysis proceeded mainly through HO• and NO₂• attack, leading to the formation of a series of intermediate products including hydroxylated, nitrated, nitrosated, and dimerized derivatives. The photolysis rate constants of PCMX and CP increased significantly with increasing concentration of NO₂⁻; but decreased in the presence of MRNOM likely due to light screening and radical quenching. The competitive kinetic experiments showed that the second-order rate constants between HO• and PCMX/CP are near diffusion-controlled limit. On the other hand, the second-order rate constants between NO₂• and PCMX/CP were determined to be 2.3×10^4 and 1.1×10^4 M⁻¹s⁻¹, respectively, by kinetic modeling, highlighting the potential importance of NO₂⁻ photosensitization to the attenuation of PCMX and CP in sunlit surface waters containing high concentration of NO₂⁻ (e.g., effluent-dominated surface waters). The evolution of nitrated byproduct, nitro-PCMX, was dependent on reaction conditions such as dissolved oxygen and HO• scavengers. In particular, HO• scavengers inhibited the further transformation of nitrated byproducts due to limited formation of HO•. It should be emphasized that the photolysis of NO₂⁻ generates NO₃⁻ as a less toxic final product; however, the potential formation of nitrated derivatives should be scrutinized due to their carcinogenicity and mutagenicity. Therefore, solar radiation may play a dual role in

the attenuation of trace organic contaminants and detoxification of NO₂⁻ in wastewater effluents or effluent-receiving surface waters.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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