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Photodegradation and toxicity changes of antibiotics in UV and UV/H_2O_2 process

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ABSTRACT

The photodegradation of three antibiotics, oxytetracycline (OTC), doxycycline (DTC), and ciprofloxacin (CIP) in UV and UV/H_2O_2 process was investigated with a low-pressure UV lamp system. Experiments were performed in buffered ultrapure water (UW), local surface water (SW), and treated water from local municipal drinking water treatment plant (DW) and wastewater treatment plant (WW). The efficiency of UV/H_2O_2 process was affected by water quality. For all of the three selected antibiotics, the fastest degradation was observed in DW, and the slowest degradation occurred in WW. This phenomenon can be explained by $R_{OH,UV}$, defined as the experimentally determined •OH radical exposure per UV fluence. The $R_{OH,UV}$ values represent the background •OH radical scavenging in water matrix, obtained by the degradation of para-chlorobenzoic acid (pCBA), a probe compound. In natural water, the indirect degradation of CIP did not significantly increase with the addition of H_2O_2 due to its effective degradation by UV direct photolysis. Moreover, the formation of several photoproducts and oxidation products of antibiotics in UV/H_2O_2 process was identified using GC–MS. Toxicity assessed by *Vibrio fischer (V. fischer)*, was increased in UV photolysis, for the photoproducts still preserving the characteristic structure of the parent compounds. While in UV/H_2O_2 process, toxicity increased first, and then decreased; nontoxic products were formed by the oxidation of •OH radical. In this process, detoxification was much easier than mineralization for the tested antibiotics, and the optimal time for the degradation of pollutants in UV/H_2O_2 process would be determined by parent compound degradation and toxicity changes.

1. Introduction

Trace amount of antibiotics has been detected in different compartments, e.g. sewage and surface water, in many countries [1–6]. The presence of antibiotics could threaten the function of ecosystem [7,8], increase the drug resistance of bacteria, and spread the antibiotic resistance genes [9–13]. The most dangerous effect of antibiotics is the development of multi-resistant bacterial strains in the environment that can no longer be treated with the presently known drugs. Therefore the occurrence, fate, and behavior of antibiotics in the environment have been the subject of growing concern and scientific interest. Several studies have shown that the biodegradation for certain antibiotics is low during wastewater treatment and many antibiotics have been detected in wastewater treatment plant (WWTP) effluents at concentrations as high as 6 μg/L [14]. It seems that physical-chemical technologies are indispensable for the degradation of antibiotics prior to discharge in the environment.

UV/H_2O_2 has been proposed as an effective treatment method for organic contaminants in drinking water and reclaimed water. In this process, pollutants are degraded in two ways. Some organic chemicals absorb UV light directly, causing destruction of chemical bonds and subsequent breakdown of the contaminant [15]. However, some other organic species do not degrade very quickly or efficiently by direct UV photolysis. Therefore, the addition of H_2O_2 is necessary, to degrade contaminants more efficiently [16].

Mineralization of trace organic compounds is a preferred goal when treating wastewater effluents for water reuse applications or drinking water from wastewater-impacted sources. However, during these wastewater treatments, complete mineralization is often unfeasible. Therefore, the toxicity assessment of reaction products and oxidation byproducts from UV/H_2O_2 is very important and necessary. As for the toxicity measurement based on microorganisms, it tends to fall into one of the five categories: population growth, substrate consumption, respiration, ATP luminescence and bioluminescence inhibition assays. The test species used for bioluminescence inhibition assay includes *Vibrio fischer*, *Vibrio harveyi* and *Pseudomonas fluorescens;* while those used for metabolic inhibition includes *Escherichia coli* and *Pseudomonas putida* [17]. Out of the various available bioassays, *V. fischer* based luminescent inhibition test is more sensitive, rapid, cost effective, and reproducible [18–20]. To date many studies have addressed the changes of bacterial toxicity on the degradation of antibiotics, by utilizing *V. fischeri* as a reference strain [21–25].

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The principal objective of this research was to provide an evaluation of the efficiency of UV/H_2O_2 process to degrade antibiotics in natural waters. Specific information on this efficiency was with focus on the effect of water quality. The availability of •OH radical in three different natural waters were compared by the value of $R_{OH,UV}$, which was obtained by the degradation of para-chlorobenzoic acid (pCBA) in UV/H_2O_2 process, a probe compound [26]. The selected antibiotics were oxytetracycline (OTC) and doxycycline (DTC), belonged to tetracycline antibiotics, and one of fluoroquinolone antibiotics ciprofloxacin (CIP), which commonly occurred at trace levels in the aquatic environment and presented in municipal wastewater [27–29]. In addition to the degradation efficiency studies, the formation of oxidation products in UV/H_2O_2 process was identified using GC–MS. Moreover, the toxicity changes with the degradation process of the targeted antibiotics were assessed by applying luminescent bacterium test.

2. Materials and methods

2.1. Reagents

Oxytetracycline hydrochloride (95+%) and doxycycline hyclate (98+%) were purchased from Sigma Chemical Corporation, while ciprofloxacin (98+%) was acquired from Fluka. Their molecular structures are shown in Table 1. Catalase from bovine liver (2000–5000 units/mg protein) and atrazine (99%) were also obtained from Sigma. Para-chlorobenzoic acid (pCBA) (98+%) and H_2O_2 (30% w/w) were supplied by Sinopharm Chemical Reagent Beijing Co., Ltd. HPLC grade acetonitrile and methanol were from Fisher Chemicals. The bacteria *Vibrio fischeri* T3 (*V. fischeri*) was provided as freeze-dried powder (0.5 g each bottle) by the Institute of Soil Science, Chinese Academy Sciences, Nanjing, China. All the other reagents were analytical grade and used as received. The working solutions were freshly prepared with ultrapure water (UW).

2.2. Water samples

The local surface water (SW) was collected from Miyun reservoir, Beijing. The treated water was from a local municipal drinking water treatment plant (DW) and wastewater treatment plant (WW) in Beijing. DW was obtained post sand filtration, and WW was obtained from the effluent of a secondary treatment train. Both of them were collected prior to any disinfection process. Samples were filtered through a 0.45 μm nylon membrane, and stored at 4 °C prior to use. The water quality of the three water matrices is presented in Table 2.

Table 1
Structures of the studied antibiotics.

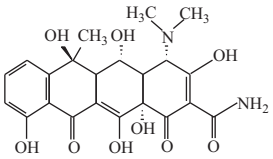
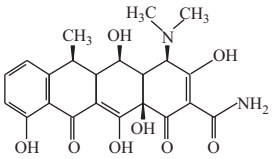
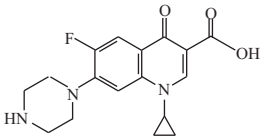
Name	Molecular formula	Chemical structure
Oxytetracycline (OTC)	$C_{22}H_{24}N_2O_9$	
Doxycycline (DTC)	$C_{22}H_{24}N_2O_8$	
Ciprofloxacin (CIP)	$C_{17}H_{18}FN_3O_3$	

Table 2
Representative water quality parameters for the waters tested.

	SW	DW	WW
pH	8.00	7.97	7.84
DOC (mg L^{-1})	4.6	3.7	5.0
A_{254} (cm^{-1})	0.0525	0.0230	0.1399
Alkalinity (mM L^{-1} as HCO_3^-)	2.85	1.50	1.58
Nitrate (mg L^{-1})	3.30	7.48	132.00
Sulfate (mg L^{-1})	42.00	34.87	60.70
Chloride (mg L^{-1})	12.40	12.61	107.30
Fluoride (mg L^{-1})	0.40	0.36	0.40

2.3. Analysis

An Agilent 1200 series HPLC system with UV/vis detector, and a ZORBAX Eclipse XDB-C_{18} column (4.6 × 150 mm, 5 μm) was used for the analysis of pCBA, atrazine and all antibiotics. The optimized mobile phase for OTC and DTC was 11% methanol–22% acetonitrile–67% 0.01 M oxalic acid solution, and the flow rate was kept at 1 mL/min. The UV/vis detector was set at 355 nm. For CIP the mobile phase was 20% acetonitrile–80% 0.01 M pH 2.5 KH_2PO_4 buffer with the flow rate 1 mL/min. The UV/vis detector was set at 278 nm.

A UV–vis spectrophotometer – U-3100 (Hitachi Co., Japan) was used to measure the aqueous solution absorbance. An ion-chromatography "Advanced Compact IC 861" (Metrohm, Switzerland) equipped with Metrosep A supp4 column (250 × 4.0 mm i.d.) was used to determine inorganic anions in SW, DW and WW. Total organic carbon (TOC) was analyzed with TOC analyzer-Phoenix 8000 (Tekmar-Dohrmann Co., U.S.A). The concentration of H_2O_2 was determined by the method of $KMnO_4$-titration [30].

HPLC-electrospray ionization (ESI)-quadrupole time-of-flight (Q-TOF)-MS/MS analysis was used to determine the photoproducts of OTC and DTC in UV and UV/H_2O_2 process. The instrument consisted of an Alliance 2695 HPLC (Waters, Manchester, United Kingdom) and a Waters Micromass Quattro Micro mass spectrometer equipped with an ESI source. Data acquisition was handled by Mass Lynx 4.0 software (Waters, Milford, MA). A XTerra MS column (4.6 × 250 mm, 5 μm), thermostatted at 40 °C, was used with a mobile phase containing water (0.2% formic acid) as eluent A and methanol (0.2% formic acid) as eluent B. Eluent A/eluent B ratios was maintained at 90/10 for the first 7 min, then changed from 90/10 (7 min) over 65/35 (15 min) to a ratio of 90/10 (16 min) which was kept constant for a final 14 min. The flow rate was kept at 0.2 mL/min. The mass spectrometer was operated in a positive ionization mode. The desolvation temperature and source temperature were set at 250 and 120 °C, respectively. The cone voltage was 25 V, and the capillary voltage was kept at 3 kV.

For the analysis of hydroxylation products in UV/H_2O_2 process, an Agilent 6890GC/5973MSD with a DB-5 MS capillary column was used. Samples (200 mL) were evaporated by freeze-dried method. Then the residue was trimethylsilylated with 0.1 mL of hexamethyldisilazane, and 0.05 mL of chlorotrimethylsilane at room temperature.

2.4. Photodegradation kinetics study

All the experiments were carried out in a reactor with a volume of 500 mL, equipped with an 11 W low-pressure Hg vapor lamp,

which emits essentially monochromatic UV light at 254 nm. A photon fluence rate of 4.5×10^{-5} E m^{-2} s^{-1} (which corresponds to a power output of 2.12 mW cm^{-2}) was obtained, using atrazine as the actinometer according to the procedures published [31]. The effective path length of the reactor was 1.9 cm determined by hydrogen peroxide actinometry [32]. Additional information about this reactor was available elsewhere [33].

UV direct photolysis and UV/H_2O_2 oxidation experiments were conducted at initial antibiotics concentration of 5 μM in buffered UW, SW, DW and WW. The parent compound degradation was monitored as a function of UV fluence (mJ cm^{-2}), which was calculated as the average photon fluence rate multiplied by the exposure time. At given time intervals, samples were withdrawn and analyzed immediately by HPLC.

A competition kinetics approach was used to determine the oxidation rate constants between the antibiotics and •OH using pCBA as a reference compound. In the experiment, H_2O_2 (1 mM) was presented in excess to ensure efficient •OH radical production, and the concentrations of pCBA and individual antibiotics in solution were 5 μM, respectively.

2.5. Toxicity assay

For the assessment of toxicity of the parent compound and its oxidation byproducts in UW, the standardized bioluminescence assay with *V. fischeri* was applied to the samples before and after exposure to UV direct photolysis and UV/H_2O_2 (H_2O_2 1 mM) oxidation at different time intervals. The concentration of antibiotics used was 50 μM. Each of the samples was carried out in triplicate. Prior to toxicity assessment, the bacteria were reactivated in 1 mL 2% NaCl solution and mixed continuously at 20 °C about 20 min. Then, they were added to culture suspension at 20 °C with continuous mixing, and cultures were harvested after 12 h, when they reached log-phase growth as determined by absorbance at 650 nm using a spectrophotometer. At this time, luminescence intensity was optimal for toxicity testing. A 1 mL aliquot of the log-phase bacterial suspension was diluted with 50 mL culture suspension to yield an absorbance of 0.40 at 650 nm. Subsequently, 1.4 mL of each treated sample and 200 μL bacterial suspensions were added to 0.4 mL of 15% NaCl solution. The decrease in bioluminescence indicated a toxic effect by degradation compounds and was measured by the Model Toxicity Analyzer (DXY-2, Institute of Soil Science, Chinese Academy Sciences, Nanjing, China) after a 15 min exposure at 20 ± 1 °C.

Positive controls, without the addition of antibiotics, consisting of bacterial suspension in 3% NaCl, the media which the test chemicals were dissolved in, were included along with the test samples. The positive controls represented the maximum *V. fischeri* growth that could be reached. Additional controls were conducted for the effect of the enzyme catalase, which was added to both the positive controls and the test samples before toxicity assays in order to destroy the produced or residual H_2O_2. In this study, the results were expressed as the percentage inhibition of luminescence in the test solutions relative to a control solution. The higher the inhibition, the more toxic was the sample.

3. Results and discussion

3.1. Effect of background water matrix

The presence of •OH radical scavengers in natural water matrices, including carbonate species (HCO_3^-, CO_3^{2-}), natural organic matter (NOM) and other organic compounds, could have negative influence on the efficiency of UV/H_2O_2. The •OH radical scavenging can be represented by $R_{OH,UV}$, defined as the experimentally determined •OH radical exposure per UV fluence for a given water matrix and initial H_2O_2 concentration [26]:

$$R_{OH,UV} = \frac{\int_0^t [\bullet OH]dt}{E_0 t} = \frac{k'_T - k'_d}{k_{OH/pCBA}} \quad (1)$$

where E_0 is the photon fluence rate which is 2.12 mW cm^{-2} in this experiment, k'_T the total observed pseudo-first order fluence-based rate constant for pCBA oxidation (cm^2 mJ^{-1}), k'_d the observed fluence-based pCBA direct photolysis pseudo-first-order initial rate constant (cm^2 mJ^{-1}), and $k_{OH/pCBA}$ is the second-order rate constant of •OH with pCBA (5×10^9 M^{-1} s^{-1}, [34]).

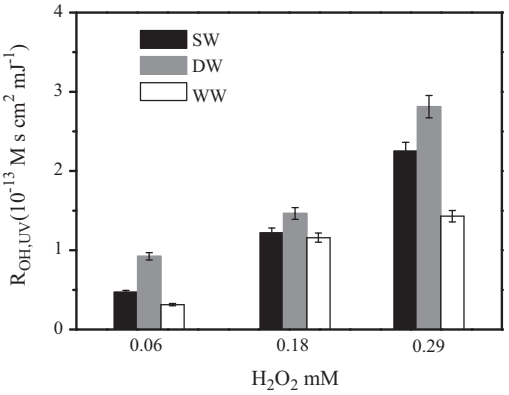


Fig. 1. $R_{OH,UV}$ values for SW, DW and WW, with 95% confidence intervals, $[pCBA]_0$ = 5 μM.

In UV/H_2O_2 process, the •OH radical availability was affected by three factors: formation rate and scavenging rate of •OH radical, and UV absorbance of the water. Since $R_{OH,UV}$ already contains a correction accounting for water absorbance, the formation rate of •OH radical based on average fluence rate has constant values for UV irradiation conditions at a given H_2O_2 concentration. Therefore, the availability of •OH radical should be only be influenced by •OH radical scavenging kinetics.

Fig. 1 shows the changes of the $R_{OH,UV}$ values in different waters. At three different initial concentrations of H_2O_2, the $R_{OH,UV}$ values were the highest in DW, and the lowest in WW. From Table 2, concentrations of dissolved organic carbon (DOC), A_{254}, nitrate, sulfate and chloride in WW were the highest, and much more •OH radicals were scavenged, leading to the lowest $R_{OH,UV}$ values. The concentrations of •OH scavengers in DW, including DOC, alkalinity, and sulfate were lower than in SW, which was the dominant factor causing a much higher $R_{OH,UV}$ value in DW than in SW. Therefore, the $R_{OH,UV}$ values could be used to compare the effect of water quality on the •OH radical exposure in different background water matrics.

3.2. UV direct photolysis and quantum yield

Table 3 presents first order rate constants (fluence-based) for the reaction of each antibiotic with UV in different waters. The direct photolysis rates of OTC and DTC decreased in natural waters compared with those in UW; while that one of CIP did not significantly change. Kim et al. [16] also reported that NOM from secondary effluent of sewage treatment plant inhibited the photochemical degradation of OTC. Doll and Frimmel [35] have reported that NOM from Lake Hohloh in the southwest of Germany enhanced the photochemical degradation of carbamazepine, indicating that NOM can act as a precursor of reactive species. NOM in SW, DW, and WW could be photoexcited to generate reactive intermediates. OTC and DTC did not react with these reactive intermediates, and they absorbed a little UV energy due to the presence of NOM, leading to the lower degradation rates. Contrarily, CIP probably reacted

Table 3
UV direct photolysis rate constants (k'_d) obtained in each of the waters tested, quantum yield (Φ), and molar absorption coefficient (ε) at 254 nm of the selected antibiotics; Correlation coefficient (R^2) given in parenthesis.

Name	k'_d (UW) $\times 10^3$ ($cm^2\ mJ^{-1}$)	k'_d (SW) $\times 10^3$ ($cm^2\ mJ^{-1}$)	k'_d (DW) $\times 10^3$ ($cm^2\ mJ^{-1}$)	k'_d (WW) $\times 10^3$ ($cm^2\ mJ^{-1}$)	Φ (mol E^{-1})	ε ($M^{-1}\ cm^{-1}$)
OTC	1.06 ± 0.01 (0.9917)	0.46 ± 0.02 (0.9925)	0.50 ± 0.03 (0.9898)	0.61 ± 0.04 (0.9915)	0.0187 ± 0.0024	13,380
DTC	1.06 ± 0.03 (0.9863)	0.42 ± 0.04 (0.9832)	0.35 ± 0.02 (0.9804)	0.40 ± 0.05 (0.9950)	0.0219 ± 0.0018	11,100
CIP	1.15 ± 0.02 (0.9685)	1.19 ± 0.01 (0.9790)	1.60 ± 0.05 (0.9979)	1.16 ± 0.03 (0.9908)	0.0134 ± 0.0032	21,970

with these reactive intermediates, resulting in a smaller change in UV direct photolysis rates.

Quantum yields (ϕ) and molar absorption are two fundamental parameters that govern the rate of direct photodegradation. The quantum yields (ϕ) were calculated using Eqs. (2)–(5) [36,37]:

$$-\frac{d[\text{antibiotics}]}{dt} = k'_d[\text{antibiotics}] = k_{s,\text{antibiotics}}\,\phi_{\text{antibiotics}}[\text{antibiotics}] \quad (2)$$

$$\phi_{\text{antibiotics}} = \frac{k'_d}{k_{s,\text{antibiotics}}} \quad (3)$$

where

$$k_{s,\text{antibiotics}} = \frac{E_p^0\,\varepsilon_{\text{antibiotics}}[1-10^{-az}]}{az} \quad (4)$$

$$a = \varepsilon_{\text{antibiotics}} \times [\text{antibiotics}] \times z \quad (5)$$

In these equations, k'_d is the pseudo-first-order direct photolysis rate constant of each antibiotics in UW (s^{-1}), $k_{s,\text{antibiotics}}$ the specific rate of light absorption by the compound (E $mol^{-1}\ s^{-1}$). E_p^0 represents the incident photon irradiance (4.5×10^{-5} E $m^{-2}\ s^{-1}$), $\varepsilon_{\text{antibiotics}}$ the decadic molar absorption coefficient at 254 nm, a the solution absorbance at wavelength 254 nm, z the optical path length (1.9 cm), and $\phi_{\text{antibiotics}}$ the quantum yield (mol E^{-1}). Table 3 also displays the decadic molar absorption coefficient ($\varepsilon_{\text{antibiotics}}$) and the quantum yield ($\phi_{\text{antibiotics}}$) of each antibiotics in UW buffered at pH 8.0.

3.3. UV/H_2O_2 process

A competition kinetics approach was used to determine the oxidation rate constants between the antibiotics and •OH using pCBA as a reference compounds [37]. By subtracting the effect of direct photolysis, the second-order rate constants of antibiotics were calculated. The experiment rate constants obtained in this study agreed well with values reported in the literature for CIP ($7.50 \times 10^9\ M^{-1}\ s^{-1}$) in our study compared to $6.22 \times 10^9\ M^{-1}\ s^{-1}$ in the study conducted by Pereira et al. [37]). The measured rate constants for OTC ($6.96 \times 10^9\ M^{-1}\ s^{-1}$), and DTC ($7.74 \times 10^9\ M^{-1}\ s^{-1}$) are comparable to the reported rate constant for tetracycline ($7.7 \pm (1.2) \times 10^9\ M^{-1}\ s^{-1}$) by Dodd et al. [38]. These rate constants were expected to be similar since tetracycline had a very similar structure to OTC and DTC.

Fig. 2 shows the photodegradation kinetics of the selected antibiotics in different waters with the addition of H_2O_2. For all compounds, the rate constants increased linearly with the applied H_2O_2. The regression equations relating the fluence-based pseudo-first order constants to the initial H_2O_2 concentration were also displayed in Fig. 2. The degradation of the three antibiotics in natural waters was slower than in buffered UW. As for the three natural waters, the oxidation of antibiotics was better for DW, followed by SW and WW, which corresponded to the trends observed for $R_{OH,UV}$

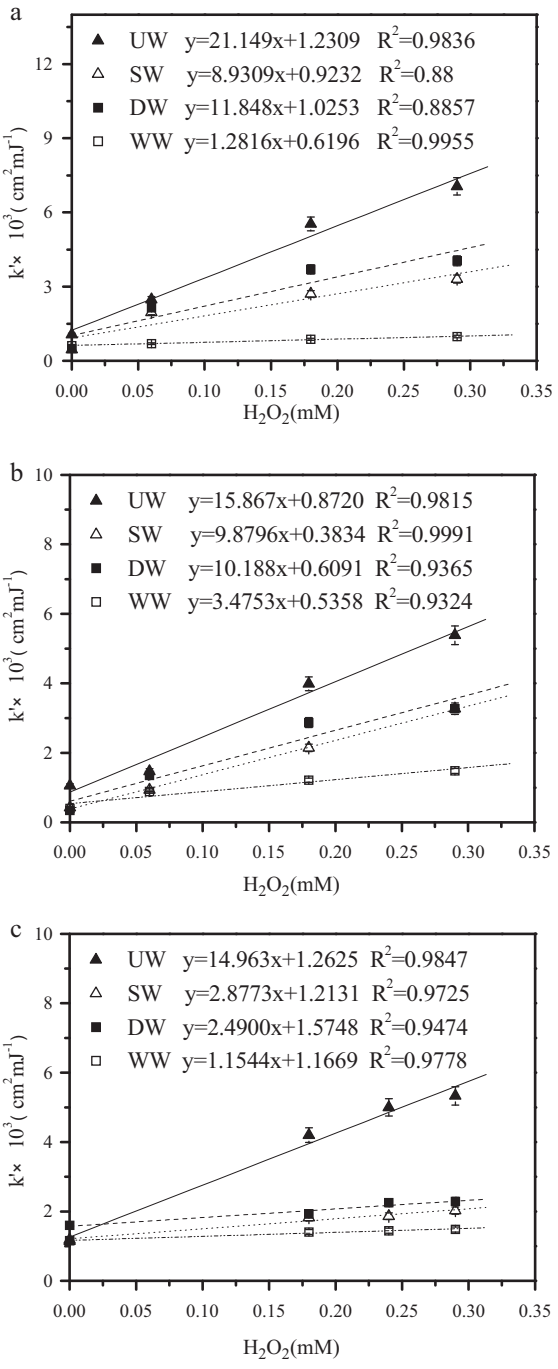


Fig. 2. Effect of background water matrix on fluence-based first order rate constant for antibiotics, $[\text{antibiotics}]_0 = 5\ \mu M$, UW buffered at pH 8.0, OTC (a), DTC (b) and CIP (c). The regression equations relating the fluence-based pseudo-first order constants to the initial H_2O_2 concentration.

values (Fig. 1). Exceptionally, for smaller $R_{OH,UV}$ value, the antibiotics degradation rates in WW did not increase substantially with the addition of H_2O_2. These results indicate that under the same UV irradiation, greater H_2O_2 dosage is required to promote greater •OH radical exposure needed to overcome the scavenging capacity of the WW and produce greater oxidation of antibiotics. It also should be noted that in natural waters, the addition of H_2O_2 was less effective in the degradation of CIP than those of OTC and DTC. This was because CIP was degraded very easily even by UV direct photolysis and •OH radical played a minor role in its degradation rates.

3.4. Toxicity assessment

Toxicity of the three antibiotics and their reaction samples were evaluated by bioluminescence inhibition using *V. fischeri*. Relative toxicity of each antibiotic in different concentrations was shown in Fig. 3. The $EC_{50,\,15\,min}$ values of OTC, DTC, and CIP were 70, 32, and 2856 mg L^{-1}, respectively. As shown in Fig. 4a, in UV photolysis, the inhibition ratio of the OTC reaction samples increased slightly when the UV fluence was less than 636 mJ cm^{-2}, then increased rapidly with the consistent increasing of UV fluence and reached to 100% after exposure to 3816 mJ cm^{-2}, and then decreased slowly with the decay of OTC. The inhibition ratio was still 76.68% when 96.26% of OTC was degraded at UV fluence of 30528 mJ cm^{-2}. Similar phenomena were found for DTC and CIP in UV photolysis. For DTC, the inhibition ratio was little increase at UV fluence less than 636 mJ cm^{-2}, and it increased from 20.76% to 87.06% after exposure to 3816 mJ cm^{-2}, and then decreased to 45.94% at UV fluence of 30,528 mJ cm^{-2}, shown in Fig. 4b. For CIP, the inhibition ratio was also little increased at UV fluence less than 636 mJ cm^{-2}, and it increased from 2.35% to 70.30% after exposure to 3816 mJ cm^{-2}, then decreased to 46.96% at UV fluence of 30,528 mJ cm^{-2} in Fig. 4c. Whereas OTC, DTC, and CIP were almost fully degraded after exposure to 30,528 mJ cm^{-2}, 22,896 mJ cm^{-2}, and 11,448 mJ cm^{-2}, no significant TOC removal were observed during the reactions. For the relatively high antibiotics concentration (50 μM) used in the toxicity assessment, a much higher UV fluence to engineering practice would be needed for the effective degradation and detoxification of the antibiotics. The result still had a certain meaning, suggesting that UV direct photolysis promoted the degradation of antibiotics but did not lead to its mineralization and detoxification.

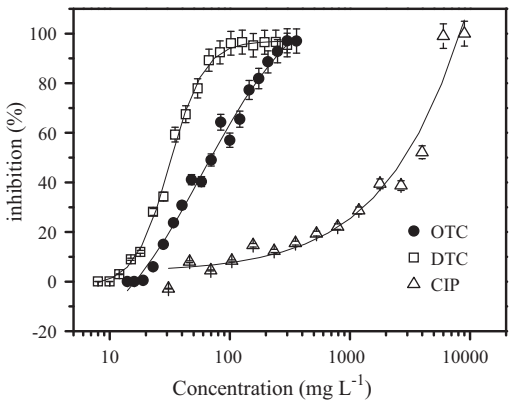
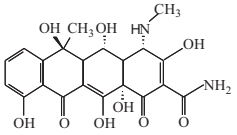
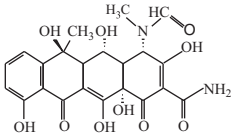
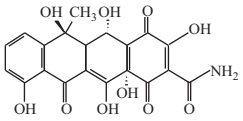
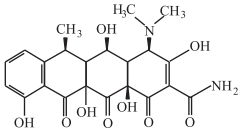
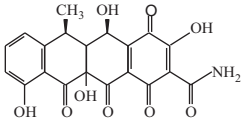
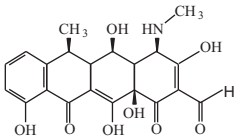


Fig. 3. Dose-response of *V. fischeri* to OTC, DTC, and CIP.

Table 4
Photodegradation products of antibiotics in UV direct photolysis identified by LC–MS/MS analysis.

Antibiotics	Retention time (min)	Molecular structure	*m/z* (relative abundance %)
OTC	3.46		224.36(50), 324.54(8), 430.69(48), 447.74(100), 483.82(22)
	11.02		233.44(25), 289.37(12), 461.47(23), 475.50(100), 497.39(10)
	13.25		186.49(35), 12.67(12), 414.69(100), 449.76(28), 491.80(10)
DTC	11.25		208.05(7), 261.35(5), 444.42(10), 461.41(100), 484.37(22)
	12.91		208.18(28), 303.47(32), 400.44(100), 463.42(46), 466.50(10)
	17.76		186.24(30), 231.30(18), 400.51(30), 416.42(100), 438.38(48)

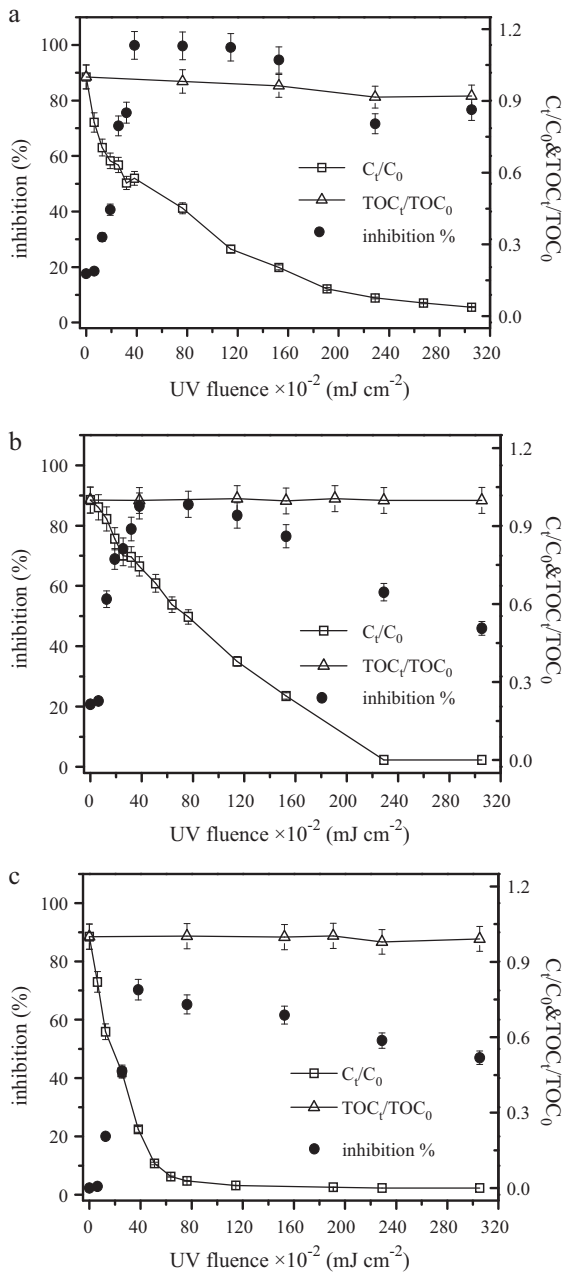
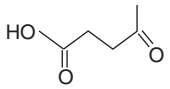
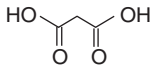
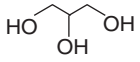
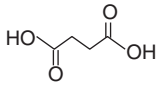
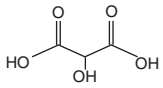
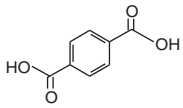
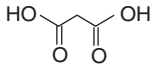
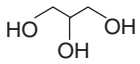
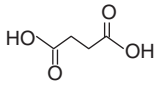
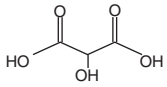
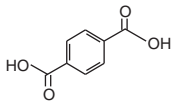
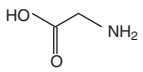
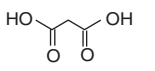
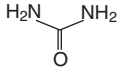
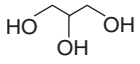
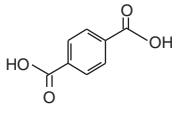


Fig. 4. Decay of antibiotics and variation of toxicity in UV direct photolysis, UW buffered at pH 8.0, [antibiotics]$_0$ = 50 μM, OTC (a), DTC (b) and CIP (c).

The antibacterial activity of tetracycline antibiotics was believed to derive from the oxygen atoms of the keto-carbonyl and hydroxyl groups, while that of fluoroquinolone was derived from the quinolone moiety [38]. HPLC-ESI-Q-TOF-MS/MS was applied to determine the photodegradation products of OTC and DTC in UV direct photolysis. As shown in Table 4, the main photolysis products from the degradation of OTC and DTC in UV photolysis still contained the characteristic structure of tetra-phenyl; however, some of their substituent groups were changed, resulting in a lower steric resistance and an easier penetration into cell of luminescent bacteria [39], which subsequently leads to the increase of toxicity. Despite the fact that the nature of the CIP-photoproducts was not assessed in this study, data available from the literatures suggested that the photodegradation of CIP would start with the loss of the piperazine ring, resulting in desethylene ciprofloxacin [40,41]; with longer irradiation time, it would be further photodegraded by the

Table 5
Oxidation products of antibiotics in UV/H_2O_2 process identified by GC–MS analysis.

Antibiotics	Name and retention time (min)	Molecular structure
OTC	4-Oxo-pentanoic acid	
	(RT = 8.89)	
	Propanedioic acid	
	(RT = 14.90)	
	Glycerin	
	(RT = 24.07)	
	Butanedioic acid	
	(RT = 26.32)	
	Hydroxymalonic acid	
	(RT = 31.70)	
	1,4-Benzenedicarboxylic acid	
	(RT = 45.68)	
DTC	Propanedioic acid	
	(RT = 14.74)	
	Glycerin	
	(RT = 23.95)	
	Butanedioic acid	
	(RT = 26.24)	
	Hydroxymalonic acid	
	(RT = 31.64)	
	1,4-Benzenedicarboxylic acid	
	(RT = 45.68)	
CIP	Glycine	
	(RT = 8.21)	
	Propanedioic acid	
	(RT = 14.77)	
	N,N′-Bis(trimethylsily)	
	(RT = 19.21)	
	Glycerin	
	(RT = 23.97)	
	1,4-Benzenedicarboxylic acid	
	(RT = 45.68)	

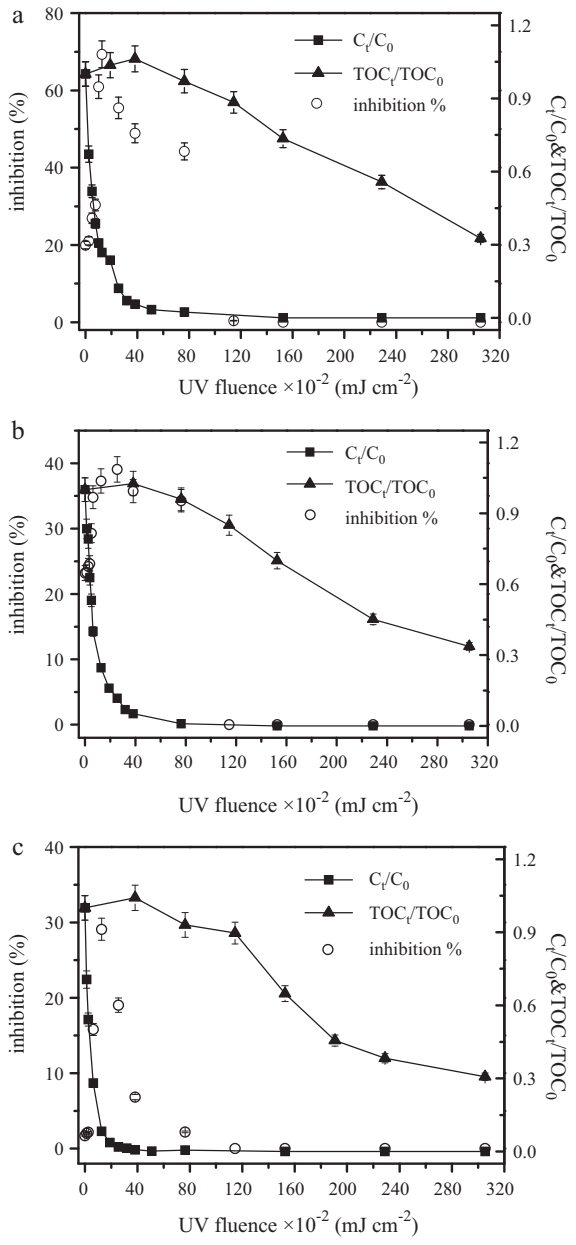


Fig. 5. Decay of antibiotics and variation of toxicity in UV/H_2O_2 oxidation process, UW buffered at pH 8.0, [antibiotics]$_0$ = 50 μM, [H_2O_2]$_0$ = 1 mM, OTC (a), DTC (b) and CIP (c).

loss of the fluorine atom [41]. These photoproducts still preserved the crucial parts of CIP, so the toxicity did not decrease.

In UV/H_2O_2 process, as shown in Fig. 5, in the first stage of the reaction, the toxicity of the byproducts greatly increased with the degradation of all three antibiotics, and then reached the maximum, when OTC, DTC, and CIP almost completely disappeared at UV fluence of 1272 mJ cm^{-2}, 2544 mJ cm^{-2}, and 1272 mJ cm^{-2}, respectively. The consistent increasing of UV fluence caused a rapid decrease in toxicity, and after exposure to certain UV fluence, there was no measurable toxicity toward *V. fischeri*. Based on the above results, the oxidation of three antibiotics could be clarified into two stages. The first stage was the degradation of the parent compounds, causing the increasing of the toxicity of the byproducts. It indicated that the character structures of OTC and DTC were not destructed in this initial period of reaction. The second stage was that these more toxic byproducts were further converted into non-toxic byproducts shown in Table 5, which were determined by GC–MS analysis, including 1, 4-benzenedicarboxylic acid, 4-oxo-pentanoic acid, propanedioic acid, hydroxymalonic acid, glycerin and some aliphatic acids etc. All of these identified compounds were unequivocally identified using the NIST98 library database with fit values higher than 90%.

As can be seen in Fig. 5, 98–99% of all three antibiotics were degraded at UV fluence of 7632 mJ cm^{-2}. However, at the same UV fluence, only 2.91%, 4.04%, and 6.98% of TOC was removed for OTC, DTC and CIP, respectively. The toxicity of reaction solution was no measurable after exposure to 11448 mJ cm^{-2}, while the removal rates of TOC were only 11.61% for OTC, 15.00% for DTC, and 10.31% for CIP. The results indicated that detoxification was much easier than mineralization for the tested antibiotics in UV/H_2O_2 process, and the optimal time for the degradation of pollutants in UV/H_2O_2 process would be determined by parent compound degradation and toxicity changes.

Results described above emphasize the need for complete evaluation of a destructive treatment, not only by following the decay of the target compound or its products, but also by measuring the toxicity of the treated water. Regarding treatment efficiency, AOPs are generally capable of completely destroying the specific antibiotics, but this is not necessarily accompanied by total mineralization. In several cases, degradation products are more biodegradable and less toxic than the original substrate, thus implying that a biological post-treatment may be feasible. This approach is applicable and based on the facts that (1) biological treatment is perhaps less costly and more environmentally friendly than any other destructive treatment, and (2) complete mineralization by AOPs induces excessive treatment costs since the highly oxidized end-products that are formed during chemical oxidation tend to be refractory to total oxidation by chemical means. These end-products which are typically represented by short carboxylic acids can, however, be degraded easily biologically.

4. Conclusion

This bench-scale study has examined the effectiveness of UV and UV/H_2O_2 treatment to degrade three antibiotics (OTC, DTC and CIP) in three natural water systems (SW, DW and WW). In UV/H_2O_2 process, the role of water quality (i.e., alkalinity, nitrate, and NOM) on •OH radical exposure can be represented by $R_{OH,UV}$ values, obtained by the photodegradation of para-chlorobenzoic acid (pCBA), a probe compound. These values can used to explain the differences in antibiotics removal among the three natural waters. Photodegradation of the three selected antibiotics yielded several organic products, and their toxic was tested applying *V. fischeri* luminescent bacterium. It showed that an increased toxic effect occurred in UV direct photolysis with the decay of the target compounds. While in UV/H_2O_2 process (H_2O_2 1 mM), the toxicity increased first, and then decreased to no measurable toxicity to the applied species. Due to the relatively high antibiotics concentration (50 μM) used, a much higher UV fluence to engineering practice would be needed for the effective degradation and detoxification of the antibiotics. In UV/H_2O_2 process, the three antibiotics were completely detoxified after exposure to 11448 mJ cm^{-2}; meanwhile their TOC removals were only about 10%. Total mineralization needed much more energy.

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