



# Photodegradation of 17 $\alpha$ -ethynylestradiol in nitrate aqueous solutions

Dong Ren, Tingting Bi, Shumei Gao, Xukun Li, Bin Huang<sup>†</sup>, Xuejun Pan

Faculty of Environmental Science and Engineering, Kunming University of Science and Technology, Kunming 650500, China

## ABSTRACT

17 $\alpha$ -Ethinylestradiol (EE2) has gotten growing concerns due to its widely detected in the environment and high estrogenic potency. However, the knowledge on the photochemical behaviors of EE2 in natural waters is still limited. Herein, the photodegradation and estrogenic potency variation of EE2 induced by nitrate were studied using a sunlight simulator consisted by a 300 W medium pressure mercury lamp and 290 nm cut-off filters. It was found that EE2 could be photodegraded at a rate of 0.0193 h<sup>-1</sup> in pure aqueous solutions, and the photodegradation of EE2 could be significantly promoted by nitrate. The photodegradation removal rate of EE2 was increased from 9% in Milli-Q water to 85% in 2.0 mM nitrate solutions. Reactive species scavenging experiments demonstrated that the photogenerated HO $\cdot$  contributed about 55% to EE2 degradation. Fe(III), Cl<sup>-</sup> and dissolved humic acid (DHA) could inhibit the photodegradation of EE2 by competing the incident light and photogenerated HO $\cdot$ , while HCO<sub>3</sub><sup>-</sup> had no influence on EE2 photodegradation. EE2 was determined to be phototransformed into organic chemicals without estrogenic potency by GC-MS and MCF-7 cell proliferation toxicity tests. These findings could extend our knowledge on the photochemical behaviors of steroid estrogens and provide information for ecological risk assessment.

**Keywords:** Ecotoxicity, Nitrate ion, Reactive oxygen species, Photochemical behavior, 17 $\alpha$ -Ethinylestradiol

## 1. Introduction

Steroid estrogens (SEs), including estrone (E1), 17 $\beta$ -estradiol (E2), estriol (E3) and 17 $\alpha$ -ethinylestradiol (EE2), have been widely detected in environmental waters [1] and gotten increasing focus in recent years due to their detrimental effects on both human and wildlife endocrine systems [2, 3]. As an active ingredient of oral contraceptives, EE2 has been extensively used and discharged into natural waters through the effluents of sewage treatment plants [4-6]. EE2 was documented to be not only more stable than natural estrogens (E1, E2 and E3) in the natural waters but also of the strongest estrogenicity among the SEs [7]. Therefore, knowledge on the behaviors and fate of EE2 in waters is essential for removal treatment and ecological risk assessment.

EE2 was reported to be degradable in pure aqueous solutions under the illumination of sunlight and simulated solar light due to its light absorption band overlapping with the solar emission spectrum in the range of 280 - 320 nm [8], but this progress was very slow even under the irradiation of a 250 W high pressure mercury lamp, in which the half-life of EE2 was determined to be decades to hundreds of hours [9]. Biodegradation of EE2 in natural environment was reported to be more time-consuming than

its direct photodegradation, and the half-life of EE2 biodegradation was determined to hundreds of days under aerobic conditions [10, 11], and a longer lag stage could be expected under anaerobic conditions [12, 13]. However, our previous investigations indicated that although the amount of SEs discharged to Dianchi Lake (centered around 24 $^{\circ}$ 48'2"N, 102 $^{\circ}$ 40'17"E) by eight sewage treatment plants was 35.8 g per day, there are no significant changes in the concentration of SEs in the sediments and waters during 2009 - 2013 [4]. Thus, other removal pathways would accelerate the dissipation of EE2 from natural waters.

Photodegradation was reported to be an effective pathway for organic pollutants removing from not only waste waters but also natural aquatic systems [14-17]. Prior studies also found that EE2 could be photodegraded with a half-life of less than 2 days in the Quinsigamond Lake surface water [11], and the quick degradation was proposed to be related to indirect photodegradation mediated by dissolved components. Ubiquitous occurrence of nitrate ( $\mu$ M - mM) in aquatic systems was always introduced by domestic and industrial wastewater discharge and fertilizer application [18]. Nitrate has been reported to be photoinductive towards organic contaminants, such as atrazine [19], trichloroethylene [20], and atenolol [21]. These photodegradations were attributed to the oxida-



This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Copyright © 2016 Korean Society of Environmental Engineers

Received January 14, 2016 Accepted March 1, 2016

<sup>†</sup> Corresponding author

Email: [huangbin@kmust.edu.cn](mailto:huangbin@kmust.edu.cn)

Tel: +86-871-65392244 Fax: +86-871-65392244

tion of the photogenerated reactive oxygen species (ROS) and other reactive photooxidants [22]. However, photochemical behaviors of EE2 induced by nitrate are unknown. Meanwhile, data regarding to the estrogenic potency of EE2 phototransformed products is scarce.

Herein, this work aimed to (1) study the contribution of nitrate photolysis to EE2 dissipation from natural waters; (2) explore the influence of water characteristics and components, mainly the pH value,  $\text{HCO}_3^-$ , Fe(III),  $\text{Cl}^-$  and dissolved humic acid (DHA), on the induced photodegradation of EE2 by nitrate; (3) determine the main photodegradation products of EE2 and propose the possible pathways responsible for EE2 photodegradation in nitrate aqueous solutions; (4) evaluate the estrogenic potency variation of EE2 induced by the photolysis of nitrate. This study can extend our knowledge on the photochemical behaviors of SEs and provide information for ecological risk assessment.

## 2. Chemicals and Methods

### 2.1. Chemicals

EE2, pyridine, benzoic acid (BZA), terephthalic acid (TPA), ultra-pure NaCl, chromatographic grade isopropyl alcohol (IPA), trimethylchlorosilane (TMCS) and N,O-bis (trimethylsilyl) trifluoroacetamide (BSTFA) were purchased from Sigma Aldrich with the highest purity available. Chromatographic grade acetonitrile, methanol, acetone, n-hexane and ethyl acetate were purchased from Merck Corporation. Dissolved humic acid (DHA) was extracted from the Dianchi Lake sediments by a conventional alkaline extraction method [23]. MCF-7 cells were purchased from Kunming Institute of Zoology, China. S1640 cell culture medium and bovine serum were purchased from GIBCO CO., Ltd.  $\text{NaNO}_3$ ,  $\text{NaHCO}_3$ ,  $\text{Fe}_2(\text{SO}_4)_3$  and other inorganic reagents used in this work were of analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd., China. Milli-Q water (electric resistance  $> 18.0 \text{ M}\Omega$ ) was used throughout this work.

### 2.2. Solution Preparation

EE2 working solution was prepared by adding  $5.00 \pm 0.05 \text{ mg}$  EE2 in 1 L Milli-Q water followed by continuously stirred for 24 h at room temperature. The supernatant was collected by passing the solution through  $0.45 \mu\text{m}$  glass fibre filters (GF/F, Millipore Corp.) which were prebaked at  $450^\circ\text{C}$  for 5 h. The filtrate was stored in dark reagent bottles at  $4^\circ\text{C}$  and used within 5 days.  $\text{NaNO}_3$  aqueous stock solution ( $5.0 \text{ mM}$ ) was prepared freshly every day and stored in dark glass bottles.

DHA stock solution was obtained by dissolving 500 mg purified humic acid powder into 300 mL NaOH solution ( $0.05 \text{ M}$ ). The solution was oscillated for 24 h followed by passing through the prebaked  $0.45 \mu\text{m}$  GF/F. The pH of the filtrate was then adjusted to  $7.8 \pm 0.1$  using  $1 \text{ M}$  NaOH and  $1 \text{ M}$   $\text{H}_2\text{SO}_4$ . The prepared DHA stock solution was stored in dark glass bottles and kept at  $4^\circ\text{C}$  for use within 3 weeks, and no significant TOC content variation was found during the storage period.

### 2.3. Photodegradation Experiments

All the photodegradations were performed on an XPA-7 mer-

ry-go-round photochemical reactor (Fig. 1) with 50 mL cylindrical quartz tubes ( $\Phi = 15 \text{ mm}$ ) and magnetic stirrers (150 rpm). A 300 W medium pressure Hg lamp was used as a light source which was equipped with 290 nm light cut-off filters. The light intensity at the surface of the irradiated solutions was measured to be  $3.71 \text{ mW m}^{-2}$  at 365 nm and  $21.6 \text{ mW m}^{-2}$  for  $\lambda > 420 \text{ nm}$ , respectively. The lamp was cooled down by recirculating cooling water and turned on preliminarily for 30 min for stabilization.

To explore the photochemical behaviors of EE2 induced by nitrate, batch experiments were conducted as follows: (i) dark controls in Milli-Q water and nitrate aqueous solutions, (ii) photolyzing EE2 in Milli-Q water, (iii) photolyzing EE2 in aqueous solutions with different concentration of nitrate and pH conditions, (iv) photolyzing different concentration of EE2 in 2 mM nitrate aqueous solutions, (v) photolyzing EE2 in 2 mM nitrate solutions with i-PrOH, BZA and TPA to detect the mechanisms responsible for nitrate mediating EE2 photodegradation, (vi) photolyzing EE2 in 2 mM nitrate solutions to identify the photodegradation products and the changes of EE2 estrogenic potency. Unless otherwise specified, the initial EE2 concentration and pH value of all the tested solutions were controlled at  $1.05 \text{ mg}\cdot\text{L}^{-1}$  and  $7.8 \pm 0.1$ . Sample tubes were fixed to allow air flow in and maintained at  $25 \pm 0.5^\circ\text{C}$  in a recirculating cooling water bath. Aliquots of  $500 \mu\text{L}$  sample of each experimental system was withdrawn at desired time intervals and analyzed the EE2 residual using a high performance liquid chromatography (HPLC). All the experiments were carried out in triplicate.

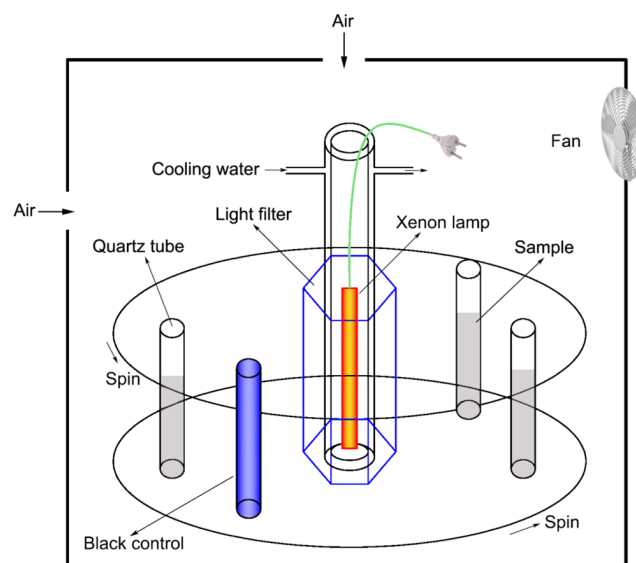


Fig. 1. Schematic diagram of the photodegradation setup.

### 2.4. Estrogenic Potency Variation Detection

MCF-7 cell proliferation toxicity experiments were conducted using a previously reported method with some modifications [24]. Briefly,  $800 \mu\text{L}$  photodegraded samples were sterilized and then diluted twice by using cell culture medium. Desired amount of starved MCF-7 cells was added with the diluted samples

(200  $\mu\text{L}$ ) and incubated at 30°C for 24 h, which was then colored and recorded the optical density at 580 nm by a plate reader (LT-4000MS, Labtech). Different concentration of EE2 standard solutions and controls were also subjected to the same estrogenic potency detection procedures to obtain a reference dose response curve.

## 2.5. Photoproducts Identification

Irradiated EE2 solutions were subjected to a solid phase extraction with the Oasis HLB cartridges (Milford, MA, USA) for obtaining the phototransformed products using our previously developed method [4]. Prior to GC-MS detection, the obtained analytes were derivatized by adding 50  $\mu\text{L}$  BSTFA + 2% TMCS and 50  $\mu\text{L}$  pyridine, and then incubated at 70°C for 30 min. The derivatized samples were separated by an Agilent DB-5 MS capillary column (30 m  $\times$  0.25 mm  $\times$  0.25 mm) using a GC (Thermo Fisher Scientific, USA) equipped with an auto-sampler Triplus AS and a DSQ quadrupole mass spectrometer. Ultra high purity helium carrier gas was maintained at a constant rate of 1 mL  $\text{min}^{-1}$ . Injector temperature was held at 280°C, and the injection volume was 1.0  $\mu\text{L}$  in a splitless mode. The column temperature for separating the targets was programmed as previously used [4]. The energy of the ionizing electrons was kept at 70 eV, and the mass spectra were operated in a full scan mode ( $m/z$ , 50 - 600).

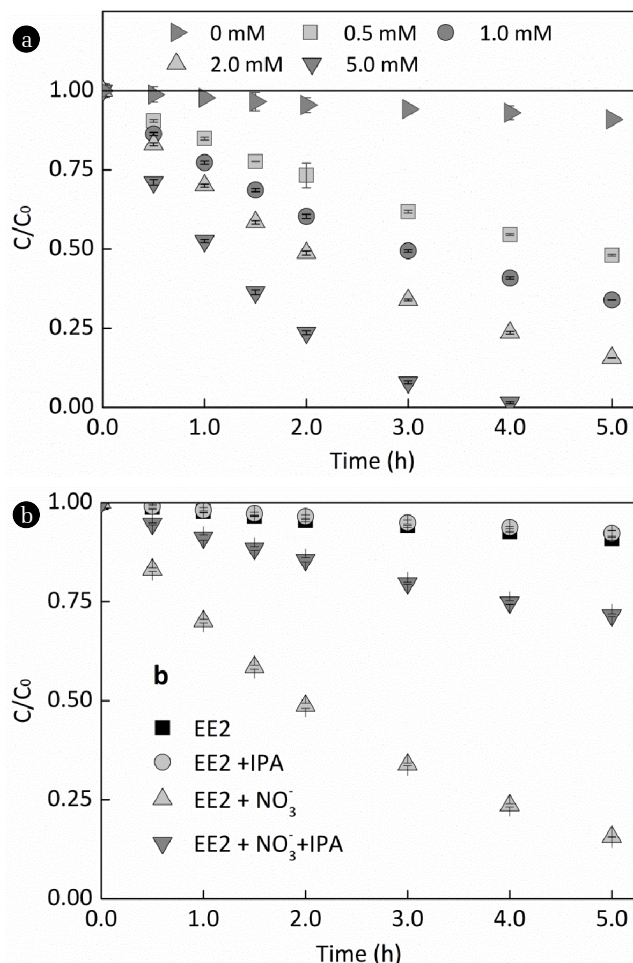
## 2.6. Procedures of EE2 Quantification

EE2 was quantified by a HPLC (Agilent Technologies 1260) equipped with a C18 reversed phase column (Waters Symmetry, 5  $\mu\text{m}$ , 4.6 mm  $\times$  250 mm) and a fluorescence detector (excitation at 236 nm, emission at 310 nm). The mobile phase was 60:40 ( $v:v$ ) of acetonitrile and Milli-Q water containing 0.1% trifluoroacetic acid. EE2 was eluted from the column by the mobile phase at a constant flow rate of 1.0 mL  $\text{min}^{-1}$ , and the retention time of EE2 was 4.9~5.1 min. The quantification limit of EE2 (LOQ) was 0.03  $\text{mg}\cdot\text{L}^{-1}$ , and the relative standard deviations (RSDs) for all samples were within 5%.

## 3. Results and discussion

### 3.1. Photodegradation of EE2 in Nitrate Solutions

Results of EE2 photodegradation in 0 - 5 mM nitrate solutions are shown in Fig. 2a. EE2 was removed about 9%, 52%, 66%, 85% and 100% from 0, 0.5, 1.0, 2.0, and 5.0 mM nitrate solutions, respectively. Compared to the photodegradations in pure aqueous solutions, nitrate could effectively accelerate the photodegradation of EE2. It was reported that nitrate could produce  $\text{HO}\cdot$  under illumination, and the yield of  $\text{HO}\cdot$  was increased linearly with the concentration of nitrate [18]. Thus, the removal of EE2 in nitrate solutions would be aroused by the photogenerated  $\text{HO}\cdot$  which can react with most organic chemicals at a diffusion-controlled rate [25]. The photodegradation of EE2 followed the pseudo-first order kinetics in both pure aqueous solutions and nitrate solutions when the removal rate of EE2 lower than 50%, and the photodegradation rate constants were determined to be 0.0193, 0.1629, 0.2540, 0.3587, and 1.1854  $\text{h}^{-1}$ , respectively. Due to the



**Fig. 2.** Photodegradation of EE2 (a) in different nitrate solutions; (b) in 2.0 mM nitrate with and without IPA.

fluctuation of the EE2 and nitrate concentration, the photodegradation of EE2 in nitrate solutions did not follow the pseudo-first order kinetics when its removal rate higher than 50%. Compared to the photodegradation of the organic pollutants shown in Table 1, EE2 is a relative photo recalcitrant chemical, and the photodegradation of EE2 always varied with different waters affected by other aquatic components.

Production of  $\text{HO}\cdot$  generated by the photolysis of 2 mM nitrate was determined by using TPA as molecular probe [33] and calculated by eq. (1) and (2). The concentration of  $\text{HO}\cdot$  was determined to be  $8.5 \times 10^{-14}$  M, which was significantly higher than that photogenerated by dissolved humic substances in natural waters [34]. Oxidation of EE2 by  $\text{HO}\cdot$  was confirmed by a well-established kinetics competition method using BZA and calculated by eq. (3) and (4) [35], which demonstrated that EE2 could react with  $\text{HO}\cdot$  at a diffusion-controlled reaction rate of  $1.14 \times 10^{-10}$   $\text{M}^{-1} \text{s}^{-1}$ . The reactions between EE2 and  $\text{HO}\cdot$  were also corroborated by using IPA (100 mM) as a selective ROS scavenger. As is shown in Fig. 2b, the removal rate of EE2 in 2 mM nitrate was decreased by 55% upon addition of IPA. Thus,  $\text{HO}\cdot$  was the main contributor to EE2 photodegradation in nitrate containing waters.

**Table 1.** Photodegradation Rate Constant (k) Comparison for Ee2 and Other Chemicals under Different Experimental Conditions

Chemicals (solute)	k (h <sup>-1</sup> )	Irradiation conditions	Source
EE2, 1.05 mg·L <sup>-1</sup> (Milli-Q water)	0.0193	300 W Hg lamp, λ > 290nm	This study
(0.5 mM nitrate)	0.1629		
(2 mM nitrate)	0.3587		
EE2, 0.6 mg·L <sup>-1</sup> (Lake water)	0.0301	Sunlight, summer sunny day	[11]
EE2, 10 μg·L <sup>-1</sup> (Distilled water)	0.094 ± 0.018	Xe lamp (250 W m <sup>-2</sup> ), λ > 300 nm	[26]
(10 mg·L <sup>-1</sup> SRFA*)	0.130 ± 0.025		
E1, 10 μg·L <sup>-1</sup> (Distilled water)	1.044 ± 0.072		
E2, 10 μg·L <sup>-1</sup> (Distilled water)	0.090 ± 0.014		
EE2, 1 ~ 2 μg·L <sup>-1</sup> (Milli-Q water)	0.08 ± 0.002	Sunlight simulator, 290 < λ < 700 nm	[27]
EE2, 10 ~ 40 mg·L <sup>-1</sup> (Distilled water)	0.61	Sunlight simulator, 300 < λ < 800 nm	[28]
(Ebre river)	0.69		
(Besos river)	0.73		
(Besos river)	0.007		
(Mediterranean sea)	0.62	Sunlight, May	
Estriol, 4 μM (Deionized water)	0.1873	150 W Xe Lamp, λ > 300 nm	[29]
(100 μM nitrate)	0.275		
Atenolol 10 μM (2 mM nitrate)	0.135	1000 W Xe lamp, λ > 290nm	[21]
Bisphenol A, 44 μM (Ultra-pure water)	0.0302	500 W Hg lamp, λ > 290nm	[30]
(1.613 mM nitrate)	0.384		
Salicylic acid, 10 mg·L <sup>-1</sup> (Milli-Q water)	0.252	500 W Xe lamp	[16]
(0.024 mM nitrate)	0.498		
Carbamazepine, 210 μM (Ultra-pure water)	0.1029	Hg lamp, λ > 290nm	[31]
(10 mg·L <sup>-1</sup> nitrate)	0.1851		
Dimethyl sulfide, 21.5 nM (25 μM nitrate)	0.12 ± 0.01	1500 W Xe lamp, 250 < λ < 700 nm	[32]

\*SRFA, Swanee River fulvic acid.

$$d[2-hTPA]/dt = k_{TPA,HO\bullet}[TPA][HO\bullet]_{SS} \quad (1)$$

$$[HO\bullet]_{SS} = d[2-hTPA]/(0.35k_{TPA,HO\bullet}[TPA])dt \quad (2)$$

$$\ln \frac{[EE2]_t}{[EE2]_0} = \frac{k_{EE2,HO\bullet}}{k_{BZA,HO\bullet}} \times \ln \frac{[BZA]_t}{[BZA]_0} \quad (3)$$

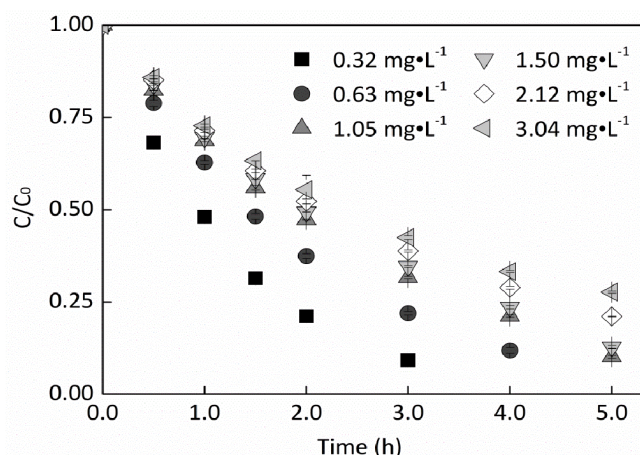
$$k_{EE2,HO\bullet} = k_{BZA,HO\bullet} \times k_{obs} \quad (4)$$

where the  $k_{TPA,HO\bullet}$ ,  $k_{BZA,HO\bullet}$  and  $k_{EE2,HO\bullet}$  are the reaction rate constant between HO• and TPA, BZA or EE2, respectively;  $k_{obs}$  is the observed degradation rate constant of EE2 in nitrate solutions; [2-hTPA], [TPA], [BZA] and [EE2] are the concentration of 2-hTPA,

TPA, BZA and EE2, respectively; the yield of 2-hTPA is 0.35; [HO•]<sub>SS</sub> is the steady state concentration of HO•.

### 3.2. Influence of EE2 Concentration

For exploring the influence of EE2 concentration on the photodegradation mediated by nitrate, different concentration of EE2 was illuminated in 2.0 mM nitrate solutions. Fig. 3 shows that the photodegradation of EE2 was significantly inhibited when its concentration increased from 0.32 mg·L<sup>-1</sup> to 1.05 mg·L<sup>-1</sup> and slightly inhibited when its concentration higher than 1.05 mg·L<sup>-1</sup>. Generally speaking, the removal rate of EE2 over an irradiation period of 3 h decreased from 91% to 58% when its concentration increased from 0.32 to 3.04 mg·L<sup>-1</sup>. This phenomenon corroborated the results



**Fig. 3.** Photodegradation of different concentration of EE2 in 2.0 mM nitrate.

reported by Zhan and his coworkers [30]. We previously found that the photodegradation rate of pure aqueous EE2 would decrease to a stable value when the concentration of EE2 increased [23], but a similar trend was not found in nitrate solutions. Furthermore, EE2 in the environment typically ranges from  $\text{ng L}^{-1}$  to  $\mu\text{g}\cdot\text{L}^{-1}$ . Therefore, the photodegradation rate of EE2 in natural waters with comparable nitrate concentration would be much faster than that determined here.

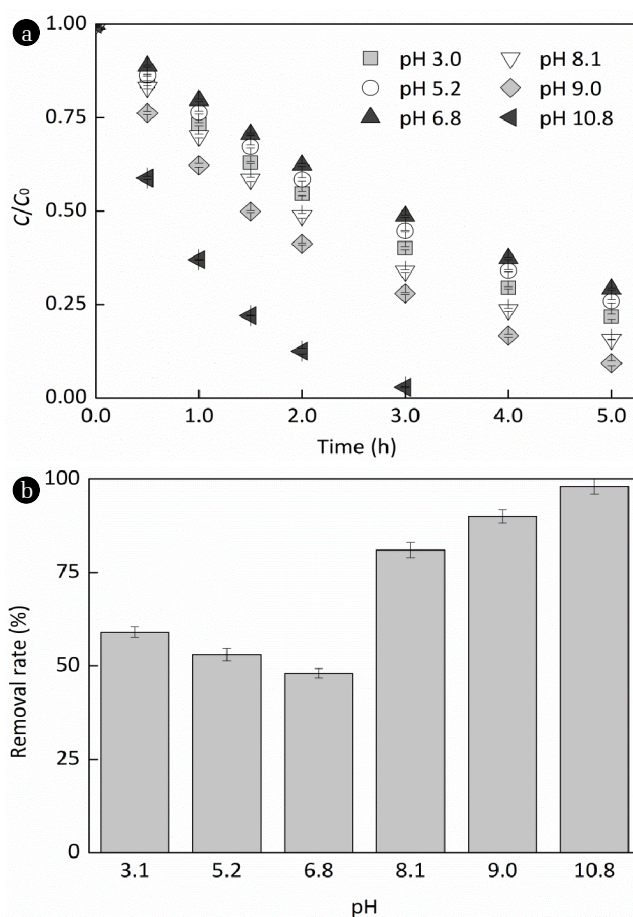
### 3.3. Influence of pH

The  $\text{pK}_a$  of EE2 was reported to be 10.5 [23], and different photodegradation rates of EE2 could be expected under different pH conditions. Thus, photodegradation of  $1.05 \text{ mg}\cdot\text{L}^{-1}$  EE2 in 2 mM nitrate solutions under different pH conditions were performed to study the influence of pH on the photodegradation kinetics of EE2.

Increasing the solution pH from 3.1 to 10.8, EE2 photodegradation rate was first decreased slightly and then increased dramatically, as shown in Fig. 4a. Removal rate of EE2 over an irradiation period of 3 h ranged from 48% to 98% (Fig. 4b), and the photodegradation rate of EE2 in alkaline solutions was always higher than that in acidic solutions. The higher degradation rate of EE2 in alkaline solutions could be typically attributed to the increased light absorption capacity [23] and the promoted yield of  $\text{HO}\cdot$  [29]. Furthermore, the delocalization of the negative charge on the phenolic hydroxyl group could increase the electron density on the benzene ring, which would be advantageous of  $\text{HO}\cdot$  attacking.

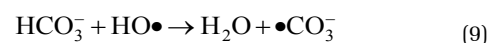
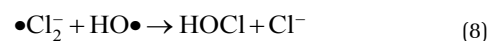
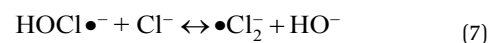
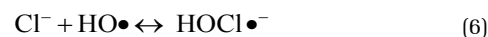
### 3.4. Influence of Coexistent Substances

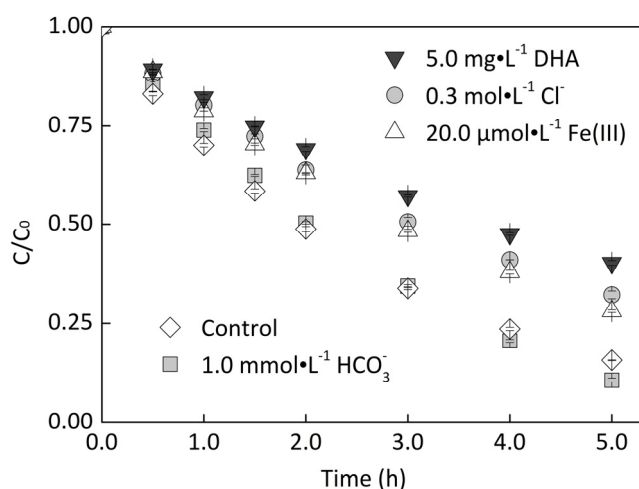
In order to explore the influence of natural water components on EE2 photodegradation, EE2 was photodegraded in 2 mM nitrate solutions in the presence of  $5.0 \text{ mg}\cdot\text{L}^{-1}$  DHA, 0.3 M  $\text{Cl}^-$ , 20  $\mu\text{M}$  Fe(III), and 1.0 mM  $\text{HCO}_3^-$ , respectively. As shown in Fig. 5,  $\text{Cl}^-$ , Fe(III) and DHA inhibited the photodegradation of EE2. The mechanisms responsible for  $\text{Cl}^-$  inhibiting EE2 photodegradation were proposed as the reactions (5) - (8) [36]. Although iron was reported to be positive in inducing some organic contaminants degradation in dissolved humic substances containing solutions [37], it inhibited the photodegradation of EE2 mediated by nitrate. The inhibition effects of Fe(III) and DHA could be attributed to the roles of light



**Fig. 4.** Photodegradation of EE2 in 2.0 mM nitrate (a) with different pH conditions; (b) removal rate of EE2 irradiated over a period of 3 h.

screening and  $\text{HO}\cdot$  scavenging [38, 39]. It was reported that  $\text{HCO}_3^-$  could inhibit  $\text{HO}\cdot$  oxidizing organic pollutants through the reaction (9) [40], but no significant influence of  $\text{HCO}_3^-$  on EE2 photodegradation was observed in this work. The possible reasons could be drawn as follows: (i)  $\cdot\text{CO}_3^-$  can selectively react with electron-rich compound, including steroid estrogens [41]; (ii) the reaction between  $\text{HO}\cdot$  and  $\text{HCO}_3^-$  can disturb the solvent-cage effect and increase the possibility of  $\text{HO}\cdot$  reacting with EE2 [42].



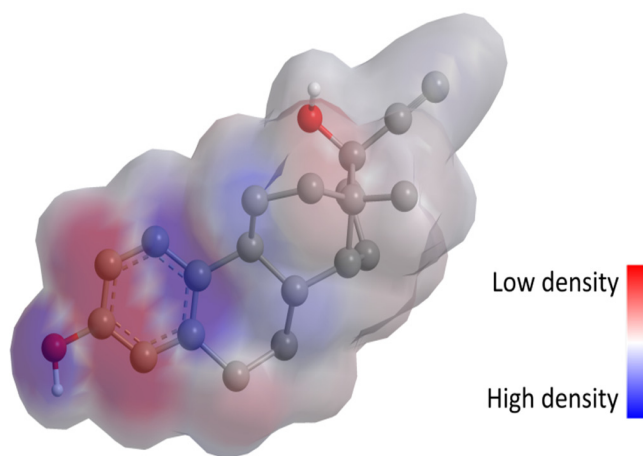


**Fig. 5.** Influence of different coexistent substances on EE2 photodegradation in 2.0 mm nitrate.

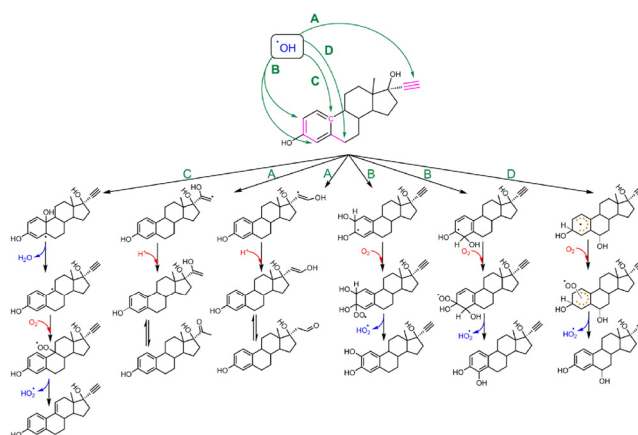
### 3.5. Photodegradation Products Identification

To further shed light on the depletion of EE2 in the irradiated nitrate solutions, the degradation products of EE2 were determined by GC-MS, and the detection results are detailed in table 2.

$\text{HO}\cdot$  is an electrophilic reagent with an electron affinity of 569.3 KJ, and it can react with organic compounds by the following three competitive pathways: (i) addition to unsaturated carbon, (ii) hydrogen abstraction from saturated carbon, (iii) electron abstraction from aromatic ring, carbon-carbon double bond or carboxylate [43].  $\text{HO}\cdot$  addition to electron rich structures is typically faster than hydrogen and electron abstraction when it reacts with organic chemicals [44]. Therefore,  $\text{HO}\cdot$  is more likely to attack the  $\pi$ -electron system in EE2. According to the GC-MS detection information (Table 2) and surface charge distribution of EE2 (Fig. 6) modeled by MM2 force field, the formation pathways regarding to the detected products were proposed in Fig. 7. The proposed mechanisms are in line with the results reported by Mazellier and his coauthors [8] and corroborate the pathways for  $\text{HO}\cdot$  oxidizing microcystin-LR [45].



**Fig. 6.** The surface charge distribution of EE2.



**Fig. 7.** Proposed pathways responsible for  $\text{HO}\cdot$  reacting with EE2.

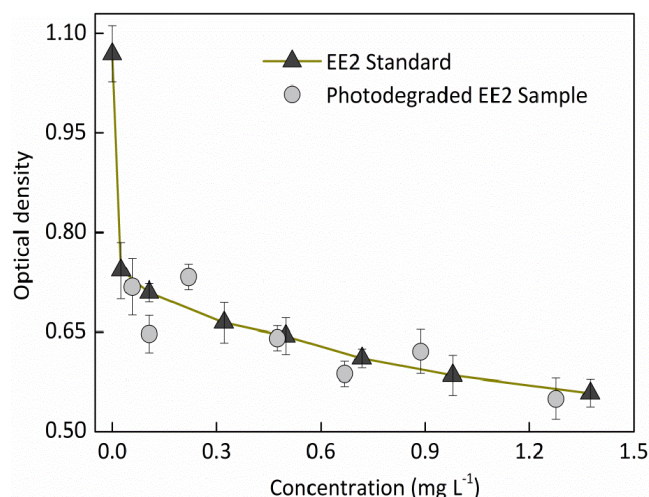
**Table 2.** GC-MS Characterization Results of EE2 Photodegradation Products

NO.	Molecular ions (m/z)	Characteristic ions (m/z)	Retention time (min)
I	440	425, 73	27.3
II	438	423, 73	28.37
III	472	457, 442, 73	29.76
IV	528	513, 73	30.8
V	528	513, 73	31.83
VI	532	502, 487, 73	32.38

### 3.6. Estrogenicity Variation of Irradiated EE2-nitrate Solutions

GC-MS usually cannot detect the volatiles and unstable species such as organic peroxides which may be decomposed on the column or the GC port. Furthermore, some products at a concentration lower than the limit of detection of GC-MS could also exhibit hazards to human health and ecosystem. Therefore, *in vitro* estrogenicity tests are essential to assess the changes in estrogenic potency of the irradiated EE2-nitrate solutions. Different EE2 concentration solutions, irradiated for 5 h previously, were subjected to the estrogenic potency determination, and the results are shown in Fig. 8.

The  $\text{OD}_{580}$  was decreased with the increased concentration of EE2 standard sample, i.e., EE2 can inhibit the proliferation of MCF-7 cell. A good agreement between the optical density of the photodegraded EE2 solutions and that of the same concentration of standard EE2 solutions was found in our study, which indicates that the photogenerated intermediates of EE2 were not estrogenic, that is to say, EE2 completely lost estrogenicity when it was photodegraded in nitrate solutions. This result corroborated the previous studies which identified the phototransformed intermediates and the lost estrogenic potency of  $17\beta$ -estradiol and EE2 in aqueous solutions [26]. Therefore, EE2 photodegradation mediated by the photolysis of nitrate is an effective way for removing EE2 from natural waters and reducing the ecotoxicity towards human and ecosystems.



**Fig. 8.** Effects of the photodegraded EE2 and EE2 standards on MCF-7 proliferation.

#### 4. Conclusions and environmental implications

Since the irradiation intensity and time used in this study were similar to the sunlight exposed by natural waters, the results of EE2 photodegradation can well simulate the photodegradation of EE2 induced by nitrate in natural waters. Although EE2 is a relative refractory chemical towards direct photolysis, it can be rapidly photo-transformed into chemicals without estrogenic potency in nitrate solutions, which was mainly aroused by the oxidization of the photo-generated HO $\cdot$ . The photodegradation of EE2 in nitrate solutions could be affected by not only the concentration of nitrate and itself but also the aquatic characteristics, including pH condition and other dissolved substances. Generally speaking, compared to EE2 biodegradation, in which the half-life of EE2 varied from 5 to 108 days [11], photodegradation mediated by nitrate is one of the fast and effective pathways for EE2 removal from sunlit natural waters.

Although the concentration of EE2 used here exceeded “natural” levels by three to six orders of magnitude, this laboratory work can be reasonably extrapolated to know the photochemical behaviors of SEs in natural waters. Photodegradation could be incorporated into sewage treatment plants to minimize the hazards of SEs to ecosystem because the effluents of sewage treatment plants were determined to be one of the main sources of estrogens in the environment.

#### Acknowledgements

This project was sponsored by the National Natural Science Foundation of China (Grant No. 41401558), Education Department Science Research Foundation of Yunnan Province, China (Grant No. 2014J022), and China Postdoctoral Science Foundation (Grant No. 2014T70887).

#### References

- Rao K, Lei B, Li N, Ma M, Wang Z. Determination of estrogens

- and estrogenic activities in water from three rivers in Tianjin, China. *J. Environ. Sci.* 2013;25:1164-1171.
- Liu R, Nelson DO, Hurley S, Hertz A, Reynolds P. Residential Exposure to Estrogen Disrupting Hazardous Air Pollutants and Breast Cancer Risk: The California Teachers Study. *Epidemiol.* 2015;26:365-373.
- Wolff SE, Veldhoen N, Helbing CC, Ramirez CA, Malpas JM, Propper CR. Estrogenic environmental contaminants alter the mRNA abundance profiles of genes involved in gonadal differentiation of the American bullfrog. *Sci. Total Environ.* 2015;521:380-387.
- Huang B, Wang B, Ren D, Jin W, Liu JL, Peng JH, Pan XJ. Occurrence, removal and bioaccumulation of steroid estrogens in Dianchi Lake catchment, China. *Environ. Int.* 2013;59:262-273.
- Ayanda OS. Occurrence, analysis, toxicity and treatment processes of pharmaceutically active compounds and hormones in water and wastewater: A review. *Int. J. Environ. Sci.* 2015;6:56-67.
- Lee DG. Removal of a synthetic broad-spectrum antimicrobial agent, triclosan, in wastewater treatment systems: A short review. *Environ. Eng. Res.* 2015;20:111-120.
- Sumpter JP, Johnson AC. 10th Anniversary perspective: reflections on endocrine disruption in the aquatic environment: from known knowns to unknown unknowns (and many things in between). *J. Environ. Monit.* 2008;10:1476-1485.
- Mazellier P, Méité L, De Laat J. Photodegradation of the steroid hormones 17 $\beta$ -estradiol (E2) and 17 $\alpha$ -ethinylestradiol (EE2) in dilute aqueous solution. *Chemosphere.* 2008;73:1216-1223.
- Liu X, Wu F, Deng N. Photodegradation of 17 $\alpha$ -ethinylestradiol in aqueous solution exposed to a high-pressure mercury lamp (250 W). *Environ. Pollut.* 2003;126:393-398.
- Jürgens MD, Holthaus KI, Johnson AC, Smith JJ, Hetheridge M, Williams RJ. The potential for estradiol and ethinylestradiol degradation in English rivers. *Environ. Toxicol. Chem.* 2002;21:480-488.
- Zuo Y, Zhang K, Zhou S. Determination of estrogenic steroids and microbial and photochemical degradation of 17 $\alpha$ -ethinylestradiol (EE2) in lake surface water, a case study. *Environ. Sci. Proc. Imp.* 2013;15:1529-1535.
- Sarmah AK, Northcott GL, Scherr FF. Retention of estrogenic steroid hormones by selected New Zealand soils. *Environ. Int.* 2008;34:749-755.
- Ying GG, Kookana RS, Dillon P. Sorption and degradation of selected five endocrine disrupting chemicals in aquifer material. *Water Res.* 2003;37:3785-3791.
- Gupta VK, Jain R, Mittal A, Saleh TA, Nayak A, Agarwal S, Sikarwar S. Photo-catalytic degradation of toxic dye amaranth on TiO $_2$ /UV in aqueous suspensions. *Mater. Sci. Eng., C.* 2012;32:12-17.
- Gupta VK, Jain R, Nayak A, Agarwal S, Shrivastava M. Removal of the hazardous dye-tartrazine by photodegradation on titanium dioxide surface. *Mater. Sci. Eng., C.* 2011;31:1062-1067.
- Liu G, Liu H, Zhang N, Wang Y. Photodegradation of salicylic acid in aquatic environment: Effect of different forms of nitrogen. *Sci. Total Environ.* 2012;435:573-577.
- Saleh TA, Gupta VK. Photo-catalyzed degradation of hazardous dye methyl orange by use of a composite catalyst consisting

- of multi-walled carbon nanotubes and titanium dioxide. *J. Colloid Interface Sci.* 2012;371:101-106.
18. Brezonik PL, Fulkerson-Brekken J. Nitrate-induced photolysis in natural waters: controls on concentrations of hydroxyl radical photo-intermediates by natural scavenging agents. *Environ. Sci. Technol.* 1998;32:3004-3010.
  19. Sharpless CM, Seibold DA, Linden KG. Nitrate photosensitized degradation of atrazine during UV water treatment. *Aquat. Sci.* 2003;65:359-366.
  20. Schlicker O, Ebert M, Fruth M, Weidner M, Wüst W, Dahmke A. Degradation of TCE with iron: the role of competing chromate and nitrate reduction. *Groundwater* 2000;38:403-409.
  21. Ji Y, Zeng C, Ferronato C, Chovelon JM, Yang X. Nitrate-induced photodegradation of atenolol in aqueous solution: kinetics, toxicity and degradation pathways. *Chemosphere* 2012;88:644-649.
  22. Mack J, Bolton JR. Photochemistry of nitrite and nitrate in aqueous solution: a review. *J. Photochem. Photobiol. A.* 1999;28:1-13.
  23. Ren D, Huang B, Bi TT, Xiong D, Pan XJ. Effects of pH and dissolved oxygen on the photodegradation of 17 $\alpha$ -ethynylestradiol in dissolved humic acid solution. *Environ. Sci. Proc. Imp.* 2016;18:78-86.
  24. Jamal JA, Ramli N, Stanslas J, Husian K. Estrogenic activity of selected Myrsinaceae species in MCF-7 human breast cancer cells. *Int. J. Pharm. Pharm. Sci.* 2012;4:547-553.
  25. Buxton GV, Greenstock CL, Helman WP, Ross AB. Critical review of rate constants for reactions of hydrated electrons, hydrogen atoms and hydroxyl radicals ( $\bullet\text{OH}/\bullet\text{O}^-$ ) in aqueous solution. *J. Phys. Chem. Ref. Data.* 1988;17:513-886.
  26. Whidbey CM, Daumit KE, Nguyen TH, Ashworth DD, Davis JC, Latch DE. Photochemical induced changes of in vitro estrogenic activity of steroid hormones. *Water Res.* 2012;46:5287-5296.
  27. Lin AYC, Reinhard M. Photodegradation of common environmental pharmaceuticals and estrogens in river water. *Environ. Toxicol. Chem.* 2005;24:1303-1309.
  28. Matamoros V, Duhec A, Albaigés J, Bayona JM. Photodegradation of carbamazepine, ibuprofen, ketoprofen and 17 $\alpha$ -ethynylestradiol in fresh and seawater. *Water Air Soil Pollut.* 2009;196:161-168.
  29. Chen Y, Zhang K, Zuo Y. Direct and indirect photodegradation of estriol in the presence of humic acid, nitrate and iron complexes in water solutions. *Sci. Total Environ.* 2013;463:802-809.
  30. Zhan M, Yang X, Xian Q, Kong L. Photochemical transformation of bisphenol A promoted by nitrate ions. *Bull. Environ. Contam. Toxicol.* 2006;76:105-112.
  31. Chiron S, Minero C, Vione D. Photodegradation processes of the antiepileptic drug carbamazepine, relevant to estuarine waters. *Environ. Sci. Technol.* 2006;40:5977-5983.
  32. Bouillon RC, Miller WL. Photodegradation of dimethyl sulfide (DMS) in natural waters: Laboratory assessment of the nitrate-photolysis-induced DMS oxidation. *Environ. Sci. Technol.* 2005;39:9471-9477.
  33. Song W, Yan S, Cooper WJ, Dionysiou DD, O'Shea KE. Hydroxyl radical oxidation of cylindrospermopsin (cyanobacterial toxin) and its role in the photochemical transformation. *Environ. Sci. Technol.* 2012;46:12608-12615.
  34. al Housari F, Vione D, Chiron S, Barbati S. Reactive photo-induced species in estuarine waters. Characterization of hydroxyl radical, singlet oxygen and dissolved organic matter triplet state in natural oxidation processes. *Photochem. Photobiol. Sci.* 2010;9:78-86.
  35. Wenk J, Von Gunten U, Canonica S. Effect of dissolved organic matter on the transformation of contaminants induced by excited triplet states and the hydroxyl radical. *Environ. Sci. Technol.* 2011;45:1334-1340.
  36. Grebel JE, Pignatello JJ, Mitch WA. Impact of halide ions on natural organic matter-sensitized photolysis of 17 $\beta$ -estradiol in saline waters. *Environ. Sci. Technol.* 2012;46:7128-7134.
  37. Lee C. Oxidation of organic contaminants in water by iron-induced oxygen activation: A short review. *Environ. Eng. Res.* 2015;20:205-211.
  38. Wenk J, Canonica S. Phenolic antioxidants inhibit the triplet-induced transformation of anilines and sulfonamide antibiotics in aqueous solution. *Environ. Sci. Technol.* 2012;46:5455-5462.
  39. Feng W, Nansheng D. Photochemistry of hydrolytic iron (III) species and photoinduced degradation of organic compounds. A minireview. *Chemosphere* 2000;41:1137-1147.
  40. Walse SS, Morgan SL, Kong L, Ferry JL. Role of dissolved organic matter, nitrate, and bicarbonate in the photolysis of aqueous fipronil. *Environ. Sci. Technol.* 2004;38:3908-3915.
  41. Espinoza LAT, Neamtu M, Frimmel FH. The effect of nitrate, Fe (III) and bicarbonate on the degradation of bisphenol A by simulated solar UV-irradiation. *Water Res.* 2007;41:4479-4487.
  42. Vione D, Khanra S, Man SC, Maddigapu PR, Das R, Arsene C, Olariu RI, Maurino V, Minero C. Inhibition vs. enhancement of the nitrate-induced phototransformation of organic substrates by the  $\bullet\text{OH}$  scavengers bicarbonate and carbonate. *Water Res.* 2009;43:4718-4728.
  43. Shah NS, Khan JA, Nawaz S, Ismail M, Khan K, Khan HM. Kinetic and mechanism investigation on the gamma irradiation induced degradation of endosulfan sulfate. *Chemosphere* 2015;121:18-25.
  44. Melloni G, Modena G, Tonellato U. Relative reactivities of carbon-carbon double and triple bonds toward electrophiles. *Acc. Chem. Res.* 1981;14:227-233.
  45. Antoniou MG, Shoemaker JA, Cruz AAdI, Dionysiou DD. Unveiling new degradation intermediates/pathways from the photocatalytic degradation of microcystin-LR. *Environ. Sci. Technol.* 2008;42:8877-8883.