



## Determination of photochemically-generated reactive oxygen species in natural water

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### Abstract

Reactive oxygen species (ROS) can be produced by interactions between sunlight and light-absorbing substances in natural water environment. ROS may participate in the indirect photolysis of trace organic pollutants, therefore resulting in changes in their environmental fates and ecological risks in natural water systems. Bisphenol A (BPA), an endocrine-disrupting chemical, exists widely in natural waters. The photodegradation of BPA promoted by ROS ( $\cdot\text{OH}$ ,  $^1\text{O}_2$ ,  $\text{HO}_2\cdot/\text{O}_2^{\cdot-}$ ), which were produced on the excitation of ubiquitous constituents (such as nitrate ion, humic substances and Fe(III)-oxalate complexes) in natural water under simulated solar radiation was investigated. Both molecular probe method and electron spin resonance (ESR) test were used for the characterization of the generated ROS. It was found that  $\cdot\text{OH}$  was photochemically produced in the presence of nitrate ions, humic substances and Fe(III)-oxalate complexes and that  $^1\text{O}_2$  was produced with the presence of humic substances. The steady-state concentrations of  $\cdot\text{OH}$  was  $1.27 \times 10^{-14}$  mol/L in a nitrate solution, and the second-order rate constant of BPA with  $\cdot\text{OH}$  was  $1.01 \times 10^{10}$  L/(mol·s).

**Key words:** reactive oxygen species; Bisphenol A; hydroxyl radicals; singlet oxygen; superoxide anion radical; photodegradation

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### Introduction

Reactive oxygen species (ROS) like hydroxyl radicals ( $\cdot\text{OH}$ ) can be generated in natural water by the interactions between sunlight and light-absorbing substances, such as nitrate/nitrite ions (Brezonik and Brekken, 1998; Zuo *et al.*, 2006a), humic substances (Sandvik *et al.*, 2000; Zuo *et al.*, 2006b), and Fe(III)/Fe(II)-oxalate complexes (Wu *et al.*, 1999; Zuo *et al.*, 2005; Deng *et al.*, 2006). These reactive intermediates as strong oxidants could induce the indirect photolysis of trace organic contaminants and therefore influence their environmental fate, persistence and ecological risk in natural water systems (Lam *et al.*, 2003; Gerecke *et al.*, 2001).

Bisphenol A (BPA) (2,2-(4,4-dihydroxydiphenyl) propane) as a representative of trace organic contaminant in natural water and a raw material for polycarbonate and epoxy resins, is an endocrine-disrupting chemical to both wildlife and human (Suzuki *et al.*, 2004). It was detected at a considerably high level in surface water (Kolpin *et al.*, 2002) and landfill leachates (Yamamoto *et al.*, 2001). Since BPA does not undergo strong light-absorption above 300 nm, its direct photolysis is unimportant in natural water. Nevertheless, sunlight-induced indirect photolysis of BPA might contribute significantly to its elimination from aquatic systems.

This article aims at the identification and determination

of the ROS ( $\cdot\text{OH}$ ,  $^1\text{O}_2$ ,  $\text{HO}_2\cdot/\text{O}_2^{\cdot-}$ ) generated from excited nitrate ions, humic substances (Suwannee River Fulvic Acid) and Fe(III)-oxalate complexes (Fe(III)-Ox) under simulated solar radiation. Besides, the potential role of ROS on the photodegradation of BPA in water was also investigated.

### 1 Experimental section

#### 1.1 Materials and chemicals

Bisphenol A and sodium nitrate were obtained from Shanghai Chemical Reagent Co., Ltd. (China).  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ,  $\text{Na}_2\text{C}_2\text{O}_4$ , methanol, and nitrobenzene were purchased from Nanjing Chemical Reagent Factory (China). Sodium azide was purchased from Tianyu Chemical Reagent Co., Ltd. (China). Suwannee River Fulvic Acid (SRFA, reference grade) was purchased from International Humic Substances Society (IHSS) (Denver, USA). Nitro blue tetrazolium (NBT) was obtained from Fluka Chemie GmbH (Buchs SG, Switzerland). Superoxide dismutase (SOD) and 5,5-dimethyl-pyrroline-N-oxide (DMPO) (purity > 97%) were obtained from Sigma-Aldrich Inc. (Louis, USA). HPLC grade acetonitrile was obtained from Tedia Ltd. (Fairfield, USA). All experimental solutions were prepared by dissolving the reagents directly in ultra pure water, which was produced from an ultrafiltration system from Labconco Corporation (Kansas, USA). All stock

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solutions were stored in a refrigerator after preparation and used within 1 month.

## 1.2 Photochemical experiments

Photochemical experiments were conducted in a NDC-2 photosimulator equipped with a 500-W medium mercury vapor lamp (Beijing Electric Light Source Institute, China) as the irradiation source. The lamp was immersed inside a borosilicate glass with a wall thickness of 4 mm, which was cooled by tap water. The borosilicate glass well filtered out the light with wavelengths below 290 nm. Fifty milliliter of each sample solution was added into a quartz test tube, which was vertically placed outside the glass well at the fixed distance. One milliliter of each reaction solution was removed at selected time intervals. Each photolysis experiment was carried out in triplicate.

## 1.3 Analytical methods

The concentrations of BPA and nitrobenzene in photochemical reaction process were measured by HPLC (Agilent1100, US) fitted with a Discovery C18 reversed-phase column (150 mm × 4.5 mm, 5 μm, Supelco, USA) at 30°C. Mobile phase composition is acetonitrile-water (60:40, V/V) at a flow rate of 1.0 mL/min. A 5-μL injection of each sample was performed in duplicate using an autosampler. Samples were analyzed by a photodiode array detector at the wavelength of 228 and 267 nm to measure BPA and nitrobenzene, respectively.

Concentrations of dissolved SRFA were expressed as total organic carbon (TOC, mg/L) and determined by a TOC analyzer (Shimadzu 2200, Japan).

Electron spin resonance (ESR) experiments were performed at room temperature using a spectrometer (EMX 10/12, Bruker, Germany) equipped with a 180-W high pressure mercury lamp. Sample in borosilicate glass capillary placed into the quartz tube was irradiated in the cavity of ESR spectrometer. The ESR tests were conducted under the following conditions: X-band, magnetic field (347.5 ± 2.5) mT, modulation amplitude 0.1 mT.

The absorbance of diformazan formed by NBT reduction by  $O_2^{\cdot-}$  was detected by UV-Vis spectrophotometer (UV-2201, Shimadzu, Japan) at wavelength 560 nm with 1 cm quartz cuvette.

Pseudo first-order kinetic constants ( $k$ ) for BPA and

nitrobenzene as molecular probe (P) in photochemical reaction were calculated by plotting  $\ln(C/C_0)$  versus irradiation time ( $t$ ), where  $C_0$  was initial concentration and  $C$  was residual concentration at irradiation time.

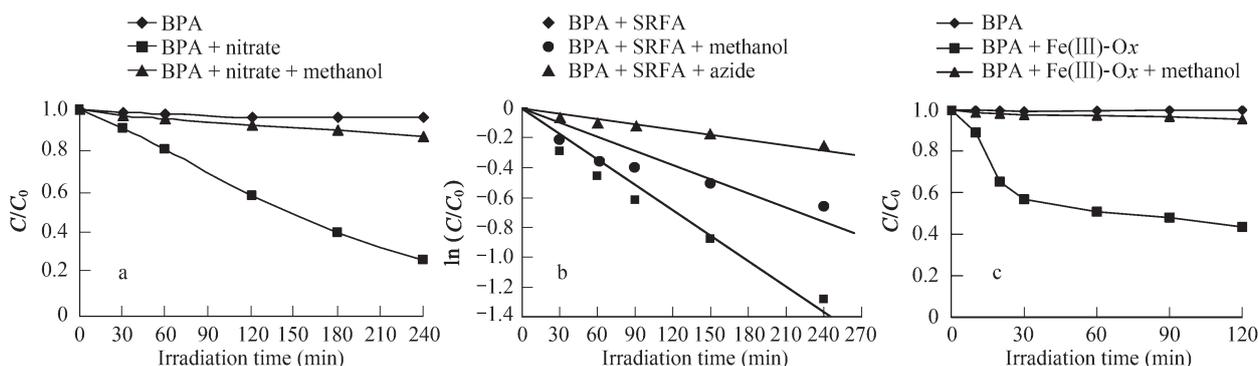
## 2 Results and discussion

### 2.1 Photodegradation of BPA in nitrate, humic substance, and Fe(III)-oxalate complexes solutions

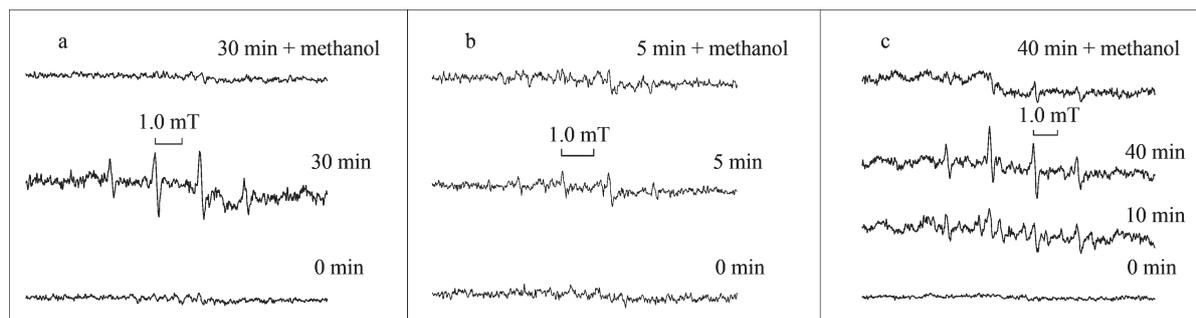
The photolysis of BPA in nitrate, humic substance (SR-FA), and Fe(III)-oxalate complexes solutions was faster than the direct photolysis of BPA in pure water. This implies that ROS-induced indirect photolysis reaction could enhance BPA photodegradation in natural water and wastewater (Fig. 1).

In order to find out whether  $\cdot OH$  was involved in the ROS-induced photodegradation of BPA, an excess amount of methanol was added as an effective  $\cdot OH$  scavenger to compete with BPA. Methanol does not absorb solar radiation and was relatively unreactive with other ROS (Zhou *et al.*, 1990). Figures 1a and 1c show that BPA degradation rates in nitrate and Fe(III)-oxalate complexes were suppressed greatly with the presence of methanol and were similar to the BPA direct photolysis rate in pure water. It implied that  $\cdot OH$ -induced BPA degradation rate was dominantly attributed to the photooxidation of  $\cdot OH$ , which was photochemically-generated from nitrates ions and Fe(III)-oxalate complexes.

To evaluate the potential roles of  $\cdot OH$  and  $^1O_2$  generated from excited humic substance on BPA photodegradation, methanol and sodium azide (Aguer *et al.*, 1996) were added in excess amount into the mixture solution of SRFA and BPA as effective scavenger for  $\cdot OH$  and  $^1O_2$ , respectively. The linear relationship between  $\ln(C/C_0)$  and irradiation time showed that the BPA photodegradation is of pseudo first-order with respect to the concentration of BPA ( $r > 0.94$ ,  $P < 0.05$ ) (Fig. 1b). The BPA photodegradation rates in SRFA solutions with the addition of methanol and sodium azide were  $5.28 \times 10^{-5}$  and  $1.97 \times 10^{-5} s^{-1}$ , respectively, which were lower than the BPA photodegradation rate in SRFA solutions ( $9.5 \times 10^{-5} s^{-1}$  by 44% and 79%). Moreover,  $^1O_2$  which was photosensitized-produced from the excitation of natural humic substances



**Fig. 1** Photodegradation of bisphenol A (BPA) in nitrate (a), humic substance (b), and Fe(III)-oxalate complexes (c) solution.  $C_{BPA} 4.4 \times 10^{-5}$  mol/L,  $C_{NO_3^-} 1.61 \times 10^{-3}$  mol/L, TOC 10 mg/L,  $C_{Fe(III)} 1.0 \times 10^{-5}$  mol/L,  $C_{Ox} 1.0 \times 10^{-4}$  mol/L,  $C_{methanol} 0.25$  mol/L,  $C_{sodium\ azide} 6 \times 10^{-3}$  mol/L.



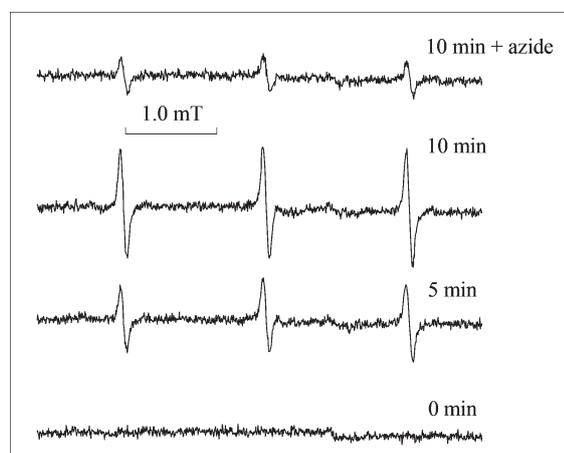
**Fig. 2** ESR spectra of DMPO-OH adduct in nitrate (a), Suwannee River Fulvic Acid (SRFA) (b) and Fe(III)-oxalate complexes (c) solution.  $C_{\text{NO}_3^-}$   $1.61 \times 10^{-3}$  mol/L, TOC 50 mg/L,  $C_{\text{Fe(III)}}$   $1.0 \times 10^{-5}$  mol/L,  $C_{\text{Ox}}$   $1.0 \times 10^{-4}$  mol/L,  $C_{\text{methanol}}$  0.25 mol/L.

(SRFA), might play a more important role on BPA photodegradation than  $\cdot\text{OH}$ .

## 2.2 Electron spin resonance determination of $\cdot\text{OH}$ and $^1\text{O}_2$

For further confirmation of  $\cdot\text{OH}$  produced upon the photolysis of nitrate ions, SRFA and Fe(III)-oxalate complexes, ESR experiments were carried out with DMPO as the spin trapper for  $\cdot\text{OH}$  (Fig. 2). The ESR signal showed a 1/2/2/1 quartet with hyperfine splitting of  $a_{\text{N}} = a_{\text{H}} = 1.505$  mT and g-factor 2.0059, which is the typical structure of the DMPO-OH adduct (Brezova *et al.*, 2004). When an excess amount of methanol was added, the ESR signal of the DMPO-OH adduct was greatly suppressed.

For further confirmation of  $^1\text{O}_2$  produced upon the photolysis of excited SRFA, ESR experiments were carried out with 4-oxo-TMP as the spin trapper for  $^1\text{O}_2$  (Moan *et al.*, 1979). Figure 3 shows that ESR signal consisting of a triplet ( $a_{\text{N}}$  1.605 mT, g-factor 2.0060) was observed by the irradiation of solutions of 4-oxo-TMP and SRFA. The signal can be attributed to the nitroxide radical 4-oxo-TEMPO (Moan and World, 1979). To confirm that the nitroxide radical was formed via  $^1\text{O}_2$  addition, sodium azide as an efficient  $^1\text{O}_2$  scavenger was added into the mixture. As expected, the ESR signal of the nitroxide radical was greatly suppressed.



**Fig. 3** ESR spectra obtained from the irradiation of SRFA solution containing 4-oxo-TMP. TOC 50 mg/L,  $C_{4\text{-oxo-TMP}}$  0.05 mol/L,  $C_{\text{sodium azide}}$  0.03 mol/L.

## 2.3 Steady-state concentration of $\cdot\text{OH}$

In order to quantify the steady-state concentration of  $\cdot\text{OH}$  ( $C_{\cdot\text{OHss}}$ ) produced from the excited nitrate ions, the competitive kinetic experiment was conducted with nitrobenzene as the molecular probe (P) (Brezonik and Brekken, 1998). In this test, the  $\cdot\text{OH}$  decay experienced the following two pathways:



Since the concentration of  $\cdot\text{OH}$  reached a steady-state under constant irradiation, the loss of probe (dP) can be expressed as:

$$-dP/dt = k_{\text{P,OH}} \times C_{\cdot\text{OHss}} \times C_{\text{P}} = k_{\text{exp}} \times C_{\text{P}} \quad (3)$$

$$\ln(C_{\text{P}}/C_{\text{P0}}) = -k_{\text{exp}} \times t \quad (4)$$

where,  $k_{\text{P,OH}}$  ( $3.0 \times 10^9$  L/(mol·s)) is the second-order rate constant for nitrobenzene with  $\cdot\text{OH}$  (Zepp *et al.*, 1987),  $k_{\text{exp}}$  ( $\text{s}^{-1}$ ) is a measured pseudo first-order rate constant, and equals  $k_{\text{P,OH}} \times C_{\cdot\text{OHss}}$ ,  $C_{\text{P}}$  (mol/L) is nitrobenzene concentration,  $t$  (s) is irradiation time.

To maintain a steady-state, BPA ( $4.4 \times 10^{-5}$  mol/L) and nitrate ion ( $1.61 \times 10^{-3}$  mol/L) were irradiated with nitrobenzene ( $7.8 \times 10^{-6}$  mol/L) as the molecular probe. A plot of  $\ln(C_{\text{P}}/C_{\text{P0}})$  vs.  $t$  yielded a straight line ( $r=0.992$ ) with the slope  $k_{\text{exp}} = 3.83 \times 10^{-5} \text{ s}^{-1}$ , and then the steady-state concentration of  $\cdot\text{OH}$  was calculated as  $1.27 \times 10^{-14}$  mol/L.

Moreover, the second-order rate constant of BPA with  $\cdot\text{OH}$  ( $k_{\text{BPA,OH}}$ ) (L/(mol·s)) was estimated from the measured pseudo first-order rate constant of BPA degradation ( $k_{\text{exp,BPA}}$ ) in nitrate solution and  $C_{\cdot\text{OHss}}$  in the same irradiation test.

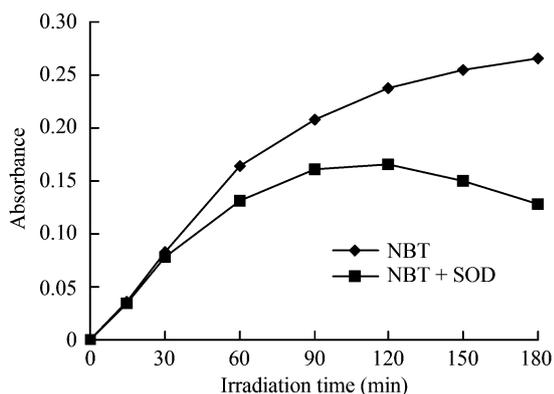
$$k_{\text{BPA,OH}} = k_{\text{exp,BPA}} / C_{\cdot\text{OHss}} = 1.01 \times 10^{10} \quad (5)$$

This result is in agreement with the value reported by Gozmen *et al.* (2003).

## 2.4 Determination of $\text{HO}_2\cdot/\text{O}_2^{\cdot-}$

The formation of superoxide anion radical ( $\text{HO}_2\cdot/\text{O}_2^{\cdot-}$ ), the first-formed ROS in the photolysis of Fe(III)-Ox complexes, was detected by NBT method (Yamakoshi *et al.*, 2003). The reduction of NBT by  $\text{O}_2^{\cdot-}$  produced

diformazan, which was quantitated in terms of the maximum absorbance at 560 nm. In addition, to examine whether the formation of diformazan was due to  $O_2^{\cdot-}$ , the effective  $O_2^{\cdot-}$  scavenger superoxide dismutase (SOD) was added to inhibit the reduction of NBT by  $O_2^{\cdot-}$ . Figure 4 shows that the amount of diformazan increased with the increasing irradiation time, but was greatly depressed with the addition of SOD, suggesting that the formation of diformazan was due to the existence of  $O_2^{\cdot-}$ .



**Fig. 4** Photoinduced generation of  $O_2^{\cdot-}$  in Fe(III)-oxalate complexes solution.  $C_{Fe(III)}$   $1.0 \times 10^{-5}$  mol/L,  $C_{Ox}$   $1.0 \times 10^{-4}$  mol/L,  $C_{NBT}$   $5.6 \times 10^{-8}$  mol/L,  $C_{SOD}$   $5.0 \times 10^{-5}$  mol/L, pH 5.0. NBT: nitroblue tereazolum.

### 3 Conclusions

The photodegradation of bisphenol A in nitrate ion, humic substances and Fe(III)-oxalate complexes solutions is faster than its direct photolysis in pure water under simulated solar radiation. This phenomenon suggests that indirect photolysis of trace organic contaminants may be enhanced by ROS-induced photooxidation in natural water. The ROS ( $\cdot OH$ ,  $^1O_2$ ,  $HO_2\cdot/O_2^{\cdot-}$ ) photochemically-generated from nitrate ion, humic substances and Fe(III)-oxalate complexes, which exist broadly in natural water system, were analyzed qualitatively and quantitatively. With the addition of effective and specific ROS scavenger such as methanol and sodium azide,  $\cdot OH$  and  $^1O_2$  were confirmed via both molecular probe method and electron spin resonance test. This study gave better understanding for the generation of ROS through the photochemical reaction of the above three common constituents in natural water under simulated solar radiation and for the potential roles of ROS on the removal of trace organic contaminants from aquatic water systems.

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