

# Generation of reactive oxygen species by promoting the Cu(II)/Cu(I) redox cycle with reducing agents in aerobic aqueous solution

Wenshu Li, Peng Zhou, Jing Zhang, Yongli Zhang, Gucheng Zhang, Yang Liu and Xin Cheng

## ABSTRACT

This study investigated the generation of reactive oxygen species (ROS) ( $O_2^{\bullet-}$ ,  $H_2O_2$ , and  $HO^{\bullet}$ ) by promoting the Cu(II)/Cu(I) redox cycle with certain reducing agents (RAs) in aerobic aqueous solution, and benzoic acid (BA) was employed as indicator for the hydroxyl radical ( $HO^{\bullet}$ ). Hydroxylamine (HA) can reduce Cu(II) to Cu(I) to induce chain reactions of copper species resulting in the generation of the superoxide radical ( $O_2^{\bullet-}$ ) and hydrogen peroxide ( $H_2O_2$ ), and the intermediate Cu(I) can further activate  $H_2O_2$  via a Fenton-like reaction to produce  $HO^{\bullet}$ , creating the remarkable BA degradation.  $O_2$  is indispensable, and unprotonated HA is the motive power in the  $O_2$ /Cu/HA system. Moreover, pH is a crucial factor of the  $O_2$ /Cu/HA system due to the protonated HA not being able to reduce Cu(II) into Cu(I). The oxidation of HA can be effectively induced by trace amounts of Cu(II), and both a higher HA dosage and a higher Cu(II) dosage can enhance  $H_2O_2$  generation and BA degradation. In addition, some other RAs that can reduce Cu(II) into Cu(I) could replace HA in the  $O_2$ /Cu/HA system to induce the generation of these ROS in aerobic aqueous solution.

**Key words** | copper, hydroxylamine, hydroxyl radical, oxygen, peroxide, reducing agents

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## INTRODUCTION

Advanced oxidation processes (AOPs) based on Fenton and Fenton-like reactions have fascinated practitioners of water treatment as effective methods to degrade refractory organic contaminants in recent years (Ganiyu *et al.* 2018). Some highly reactive oxidants such as the hydroxyl radical ( $HO^{\bullet}$ ) can be produced during the decomposition of hydrogen peroxide ( $H_2O_2$ ) via the catalytic redox cycle of Fe(III)/Fe(II). Similar to the circulation of Fe(III)/Fe(II), copper can also decompose  $H_2O_2$  into  $HO^{\bullet}$  via the Fenton-like copper redox (Pham *et al.* 2013). Previous studies have reported that cuprous copper [Cu(I)] is a strong oxygen scavenger and some reactive oxygen species (ROS) were produced during the redox reactions of Cu(I), such as the superoxide radical ( $O_2^{\bullet-}$ ) and  $H_2O_2$  (Wen *et al.* 2014; Zhou *et al.* 2016a; Zhou *et al.* 2018). Then, a sequence of reactions between the ROS and other components in aerobic aqueous solution would be induced (Yuan *et al.* 2012). As a further product,  $HO^{\bullet}$  can be produced via a Fenton-like reaction between Cu(I) and  $H_2O_2$  (Yuan

*et al.* 2012; Lee *et al.* 2016; Zhang *et al.* 2017). It is remarkable that Cu(I) is a well-known activator of  $H_2O_2$  to produce  $HO^{\bullet}$ , whereas Cu(II) was seldom investigated to activate  $H_2O_2$  due to its instability and naturally occurring copper being mostly in the cupric copper [Cu(II)] oxidation state.

However, it has been proposed that the oxygen-dependent oxidation of 1,4-hydroquinone undergoing copper-redox reactions can produce ROS such as  $O_2^{\bullet-}$  and  $H_2O_2$  (Yuan *et al.* 2013), and the further generation of  $HO^{\bullet}$  has been proved based on electron spin resonance (ESR) spectroscopy (Lee *et al.* 2016). Nonetheless, the product  $HO^{\bullet}$  gained little attention and the previous literatures mainly focused on its biological effects, such as oxidative DNA damage (Mobley *et al.* 1999; Kim *et al.* 2015). In summary, the capacity of 1,4-hydroquinone to reduce Cu(II) into Cu(I) is the decisive prerequisite to induce the chain reactions of copper species and accelerate the Cu(II)/Cu(I) redox cycle.

Therefore, some reducing agents (RAs), which are capable of reducing Cu(II) into Cu(I), can be quickly oxidized in aerobic aqueous solutions and undergo copper-redox reactions. More importantly, several ROS ( $O_2^{\cdot-}$ ,  $H_2O_2$ , and  $HO^{\cdot}$ ) which can oxidize organics and inorganics in aqueous solution could be produced during these oxidation processes. Hydroxylamine (HA) is a distinguished reducing agent and was commonly considered as an antioxidant for reactive oxidants (Krishna *et al.* 1998). Recently, HA was introduced into some metal-catalyzed AOPs to enhance the production of reactive radicals (e.g.  $HO^{\cdot}$  and  $SO_4^{\cdot-}$ ) by accelerating the Fe(III)/Fe(II) and Cu(II)/Cu(I) redox cycles (Chen *et al.* 2011; Zou *et al.* 2013; Zhou *et al.* 2016b; Hou *et al.* 2017). On the strength of the strong reducibility, in this study, HA and some other RAs were employed to induce the chain reactions of oxygen species for generating  $HO^{\cdot}$  and some other ROS without the external supply of  $H_2O_2$ .

Thus, the objective of this study was to evaluate the degradation of organic contaminants in the  $O_2$ /Cu/HA system while benzoic acid (BA) was employed as the target organic pollutant. The generation of ROS ( $O_2^{\cdot-}$ ,  $H_2O_2$ , and  $HO^{\cdot}$ ) by promoting the Cu(II)/Cu(I) redox cycle with HA in aerobic aqueous solution was investigated, and mainly focused on the mechanism of the ROS ( $O_2^{\cdot-}$ ,  $H_2O_2$ , and  $HO^{\cdot}$ ) generation and the effect of pH. The effects of HA dosage and Cu(II) dosage on  $H_2O_2$  generation and BA degradation in the  $O_2$ /Cu/HA system were also explored. Moreover, in order to expand the common mechanism of the  $O_2$ /Cu/RAs system, some RAs (e.g. *L*-ascorbic acid, *N*-methylhydroxylamine, *p*-hydroquinone, and sodium nitrite) were introduced into the  $O_2$ /Cu/RAs system to replace the role of HA in the  $O_2$ /Cu/HA system.

## EXPERIMENTAL SECTION

### Materials

HA ( $\geq 99.0\%$ ), copper sulfate pentahydrate ( $\geq 99.0\%$ ), BA ( $\geq 99.5\%$ ), and nitro blue tetrazolium (NBT,  $\geq 98.5\%$ ) were supplied by Sigma-Aldrich. Neocuproine hemihydrate (NCP,  $\geq 98\%$ ), coumarin ( $\geq 99.5\%$ ), 7-hydroxycoumarin ( $\geq 99.0\%$ ), acetone oxime ( $\geq 98.0\%$ ), and methyl tert-butyl ether (MTBE,  $\geq 99.0\%$ ), and 5,5-Dimethyl-1-pyrroline N-oxide (DMPO,  $\geq 98.0\%$ ) were purchased from the Aladdin Industrial Corporation. *N*-methylhydroxylamine (NMHA,  $\geq 99.0\%$ ), *L*-ascorbic

acid (*L*-AA,  $\geq 99.7\%$ ), *p*-hydroquinone (*p*-HQ,  $\geq 99.0\%$ ), sodium nitrite (SN,  $\geq 99.0\%$ ), *tert*-butyl alcohol (TBA),  $H_2O_2$  (30%, v/v), acetone, and potassium titanium oxalate were purchased from the Sinopharm Chemical Reagent factory. Ammonium acetate and methanol were of HPLC grade and supplied by Sigma-Aldrich. The other chemical reagents in these experiments were all of analytical grade.

### Experimental procedures

For experiments requiring aerobic or anoxic condition, pure  $O_2$  ( $\geq 99.2\%$ ) or pure  $N_2$  ( $\geq 99.99\%$ ) was bubbled into the reactor for 10 min prior to the initiation of the reaction and throughout the entire experiment at  $25 \pm 1^\circ C$ . Some other experiments were performed in a 250 mL sealed Florence flask with a constant stirring rate at  $25 \pm 1^\circ C$  to explore the consumption of DO in the aqueous solution. Each reactive aqueous solution was prepared with deionized water, the desired dosages of Cu(II) and BA or coumarin were firstly added into the reaction solution, and then adjusted to the desired pH by phosphoric acid-phosphate buffering. The reaction was started by adding a small volume of the desired fresh RAs solution. To analyze the concentrations of  $H_2O_2$ , BA, and Cu(I), the samples were withdrawn from the reaction solution and mixed with excess potassium titanium oxalate, sodium sulfite, and NCP at predetermined time intervals, respectively. Moreover, NBT was introduced into the  $O_2$ /Cu/HA system to verify the generation of  $O_2^{\cdot-}$  through the qualitative detection of monoformazan, which is the characteristic product of the reaction between NBT and  $O_2^{\cdot-}$ .

To distinguish the contribution of  $HO^{\cdot}$  on BA degradation, the excess TBA was added to the reaction solution to perform the quenching experiment. Resulting from that the 7-hydroxycoumarin was the characteristic product of the reaction between coumarin and  $HO^{\cdot}$ , coumarin was added into the  $O_2$ /Cu/HA systems to act as a  $HO^{\cdot}$  catching agent to identify the generation of  $HO^{\cdot}$  through detecting 7-hydroxycoumarin. All experiments were independently repeated at least 3 times, and the average values along with one standard deviation (error bars) are provided in the figures.

### Analysis methods

The BA concentration was measured by high performance liquid chromatography (HPLC, Waters e2695) equipped

with a 2,489 nm UV absorbance detector and a reverse-phase C18 column (4.6×150 mm). The mobile phase composition and flow rate were methanol/0.1% ammonium acetate (40:60, v/v) and 1 mL/min, respectively, and the detection wavelength was 227 nm. The concentration of 7-hydroxycoumarin was analyzed by a HPLC (Waters e2695) equipped with a 2475 fluorescence (FLR) detector and a reverse-phase C18 column (4.6×150 mm). The mobile phase composition and flow rate were methanol/0.1% ammonium acetate (50:50, v/v) and 1 mL/min, respectively. The fluorescence intensity of 7-hydroxycoumarin was measured using a 2475 FLR detector with excitation wavelength of 346 nm and detection wavelength of 456 nm. The pH and DO concentration were measured by a pH meter (Shanghai Leici Apparatus Fac., China) and a DO meter (JPB-607A), respectively. The  $O_2^{\cdot-}$  was identified by the NBT method (Liu et al. 2009; Cheng et al. 2017) on a UV-vis spectrometer (UV-1800, MAPADA). The concentrations of Cu(I) and  $H_2O_2$  were determined by spectrophotometric methods on a UV-vis spectrometer (UV-1800, MAPADA), which is described in detail in Text S1 (available with the online version of this paper). HA was derived to acetone oxime by reaction with acetone and extracted by MTBE, and then measured by gas chromatography (QP2010 Plus) using a FID detector (Zou et al. 2013). Electron spin resonance (ESR, Bruker EMX plus) experiments were performed using DMPO as a spin-trapping agent to identify the generation of  $HO^{\cdot}$  (Text S2, available online).

## RESULTS AND DISCUSSION

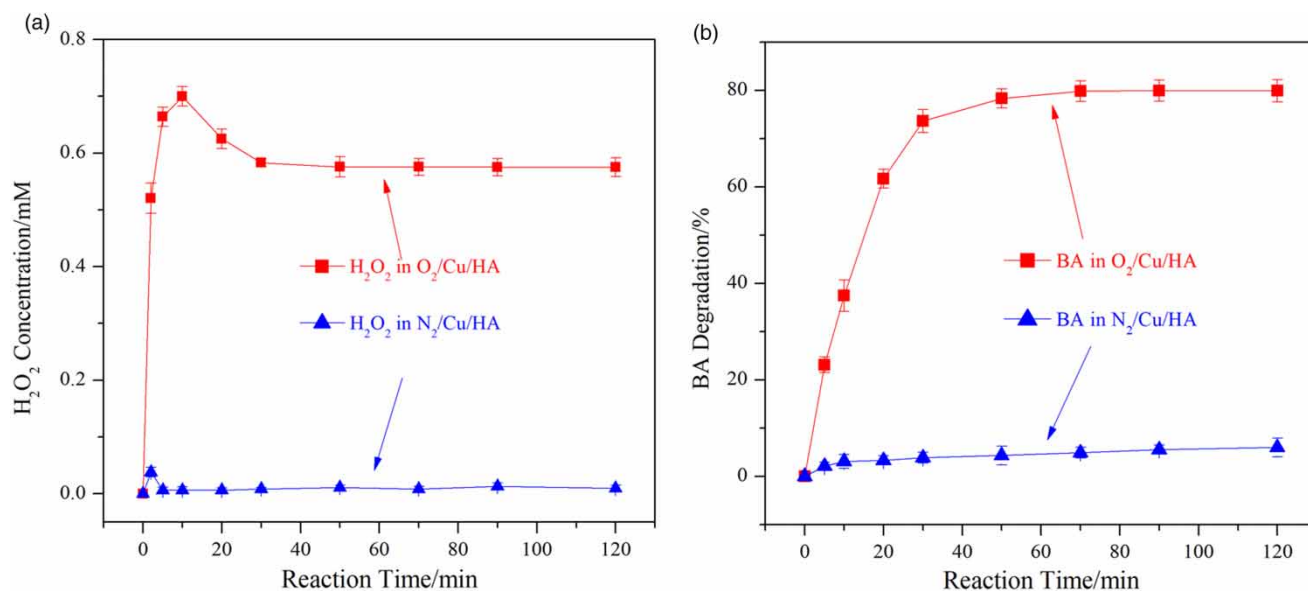
### $H_2O_2$ generation and BA degradation in the $O_2/Cu/HA$ system

The generation of  $H_2O_2$  and the degradation of BA during the copper-catalyzed oxidation of HA in aerobic and anoxic aqueous solutions was investigated. As shown in Figure 1(a), the generation of  $H_2O_2$  was significant in the  $O_2/Cu/HA$  system, and the  $H_2O_2$  concentration became relatively stable after an initial rise and later drop. With the addition of 2 mM HA in the aerobic aqueous solution catalyzed by copper, the concentration of  $H_2O_2$  was 0.58 mM at 120 min. Moreover, as a reactive radical probe, 79.9% BA was degraded in the  $O_2/Cu/HA$  system as shown in Figure 1(b). On the contrary, the generation of  $H_2O_2$  was seriously weakened in anoxic aqueous solution (the  $N_2/Cu/HA$  system), resulting in less than 4% BA being degraded at 120 min, which shows that  $O_2$  is indispensable in the  $O_2/Cu/HA$  system.

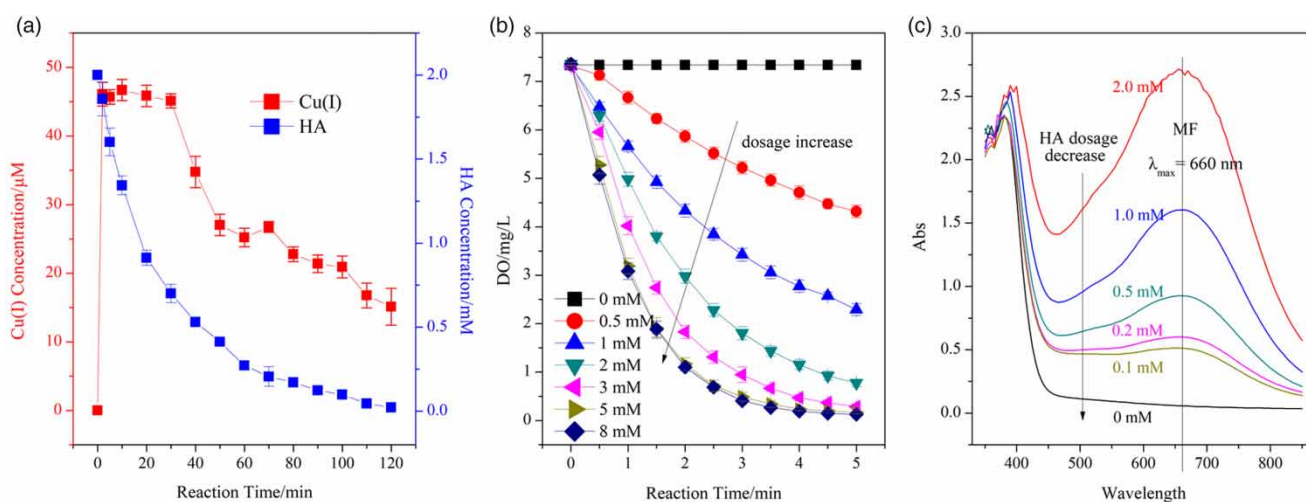
### Mechanism discussion

#### HA reduce Cu(II) into Cu(I)

As shown in Figure 2(a), HA was gradually consumed in the  $O_2/Cu/HA$  system, the residual HA was only 0.02 mM at 120 min. With the consumption of HA, Cu(I) was rapidly generated in the  $O_2/Cu/HA$  system, and almost all Cu(II)

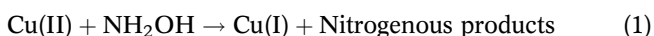


**Figure 1** | Generation of  $H_2O_2$  (a) and BA degradation (b) in the  $O_2/Cu/HA$  system. pH = 7 ± 0.2,  $[HA]_0 = 2$  mM,  $[Cu(II)]_0 = 50$  μM,  $N_2$  flow rate 0.6 L/min (anoxic) or  $O_2$  flow rate 0.3 L/min (aerobic), and  $[BA]_0 = 10$  μM.



**Figure 2** | Cu(I) generation and HA consumption (a), DO consumption in a sealed Florence flask (b), UV-vis spectra of MF (c) in the O<sub>2</sub>/Cu/HA system. pH = 7 ± 0.2, [Cu(II)]<sub>0</sub> = 50 μM, [BA]<sub>0</sub> = 10 μM, (a) O<sub>2</sub> flow rate 0.3 L/min, [HA]<sub>0</sub> = 2 mM; (b) [HA]<sub>0</sub> = 0, 0.5, 1, 2, 3, 5, 8 mM, [DO]<sub>0</sub> = 7.34 ± 0.06 mg/L in a sealed Florence flask; (c) O<sub>2</sub> flow rate 0.3 L/min, [HA]<sub>0</sub> = 0, 0.1, 0.2, 0.5, 1.0, 2.0 mM, [NBT]<sub>0</sub> = 0.5 mM.

was transformed into Cu(I) in the initial 25 min. As a strong reducing agent, HA can accelerate the reduction of Cu(II) to Cu(I) (Equation (1)) so as to initiate the chain reactions of the copper species (Kim *et al.* 2015; Zhou *et al.* 2016a).



### Consumption of O<sub>2</sub> and generation of O<sub>2</sub><sup>-•</sup>

Then, as a strong scavenger of O<sub>2</sub>, Cu(I) can react with O<sub>2</sub> to produce O<sub>2</sub><sup>-•</sup> (Equation (2)) (Yuan *et al.* 2012; Yuan *et al.* 2013; Zhou *et al.* 2016a). The effect of HA dosage on DO consumption was then investigated in a sealed Florence flask at initial 5 min to investigate the role of O<sub>2</sub> in the O<sub>2</sub>/Cu/HA system. Figure 2(b) specifically demonstrates that DO was clearly consumed and the consumption rate of DO (defined over the initial 2 min) increased with the growth of HA dosage. The DO consumption rates were 19.72, 41.53, 60.42, 74.72, 86.53, and 86.81 (μM/min), respectively. Thus, the sealed experiments clearly indicate the DO consumption in the O<sub>2</sub>/Cu/HA system.

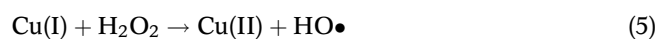
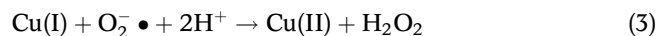


Moreover, the previous studies (Liu *et al.* 2009; Cheng *et al.* 2017) showed that the reduction reaction between O<sub>2</sub><sup>-•</sup> with NBT and the inherent spectral characteristics of its reduction product, monoformazan (MF), provide a universal detection and quantitation method for O<sub>2</sub><sup>-•</sup> that has

high sensitivity. Therefore, NBT was introduced into the reaction solution to verify the generation of O<sub>2</sub><sup>-•</sup> in the O<sub>2</sub>/Cu/HA system. As shown in Figure 2(c), the spectra clearly illustrate a peak at 660 nm, which is the characteristic absorption peak of MF. The absorbance of the characteristic reaction product (MF) increased gradually with the growth of the HA dosage. Thus, it reveals the generation of O<sub>2</sub><sup>-•</sup> in the O<sub>2</sub>/Cu/HA system.

### Generation of H<sub>2</sub>O<sub>2</sub> and HO<sup>•</sup>

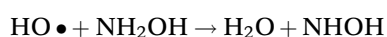
The intermediate Cu(I) can react with O<sub>2</sub><sup>-•</sup> to induce the generation of H<sub>2</sub>O<sub>2</sub> (Equation (3)) (Yuan *et al.* 2012; Yuan *et al.* 2013; Zhou *et al.* 2016a) in aerobic aqueous solution, which is probably responsible for the strong generation of H<sub>2</sub>O<sub>2</sub>, as shown in Figure 1(a). As an excellent activator for H<sub>2</sub>O<sub>2</sub>, Cu(I) can react with intermediate H<sub>2</sub>O<sub>2</sub> via a Fenton-like reaction (Equation (5)) (Yuan *et al.* 2013; Zhou *et al.* 2016b) to continuously induce the generation of HO<sup>•</sup>, resulting in H<sub>2</sub>O<sub>2</sub> consumption and BA degradation.



Based on the high reaction rate constant ( $k = 6.0 \times 10^8 \text{ M}^{-1}\text{s}^{-1}$ ) with HO<sup>•</sup> (Yang *et al.* 2018), TBA was always used as the HO<sup>•</sup> quencher in AOPs. Thus, in this study, TBA was employed as the HO<sup>•</sup> quencher to identify the



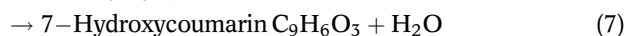
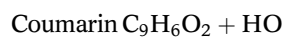
contribution of BA degradation by HO• in the O<sub>2</sub>/Cu/HA system. Figure 3(a) shows the effect of TBA on H<sub>2</sub>O<sub>2</sub> generation and BA degradation in the O<sub>2</sub>/Cu/HA system. The addition of 30 mM TBA almost completely inhibited the BA degradation, and less than 4% BA was degraded at 120 min when the dosage of HA was 2 mM. Furthermore, the generation of H<sub>2</sub>O<sub>2</sub> in the O<sub>2</sub>/Cu/HA-TBA system is stronger than that in the O<sub>2</sub>/Cu/HA system. As a reducing agent, HA can be degraded by the generated HO• via Equation (6) (Buxton et al. 1988), which may decrease the utilization ratio of HA to reduce Cu(II) into intermediate Cu(I). On the contrary, TBA can quench HO• to increase the utilization ratio of HA in the O<sub>2</sub>/Cu/HA-TBA system.



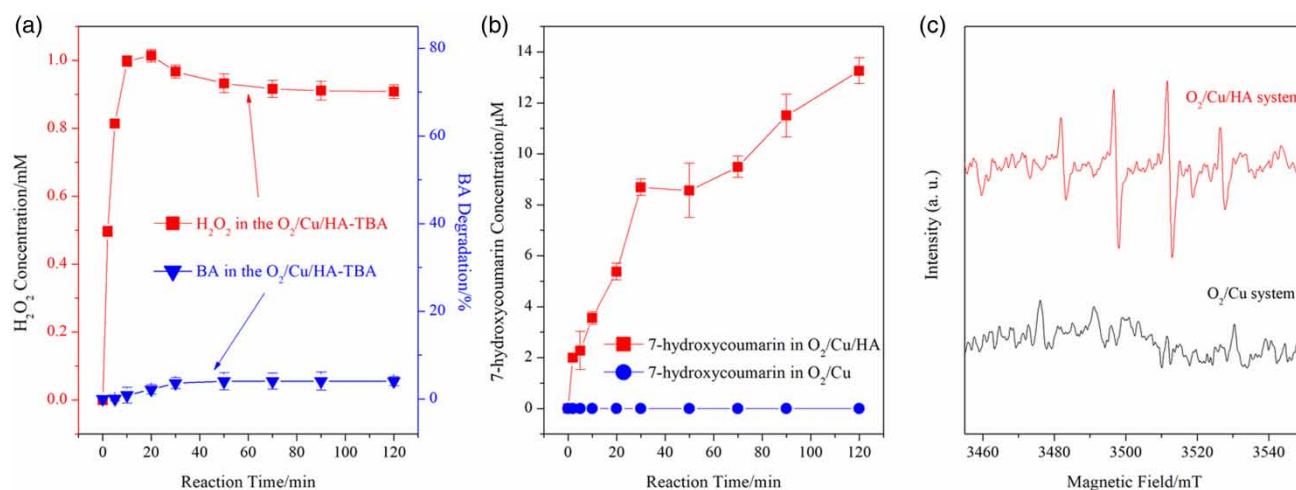
$$(\text{pH } 4.0, k < 5.0 \times 10^8 \text{ M}^{-1}\text{s}^{-1}; \text{pH } 8.0, k = 9.5 \times 10^9 \text{ M}^{-1}\text{s}^{-1}) \quad (6)$$

Due to high reactivity and extremely short lifetime of HO• (~10<sup>-9</sup> s) (Louit et al. 2005; Zhang et al. 2012), it is very hard to make the direct detection of HO• feasible. In general, HO• is qualitatively or semi-quantitatively detected by some indirect methods and several kinds of indirect HO• measurement techniques (e.g. ESR, UV/Vis absorption spectroscopy, and luminescence) have been developed in recent years (Tsai et al. 2001; Louit et al. 2005; Maezono et al. 2011; Lim et al. 2016). Therefore, an attempt at ESR experiment was conducted to detect HO• with DMPO employed as a radical trapping agent, and HO• can be detected by measuring the intensity of the DMPO-OH adduct signal (Lee et al.

2016). As shown in Figure 3(c), the specific spectrum of DMPO-OH (peak light ratio of 1:2:2:1) was obtained in the O<sub>2</sub>/Cu/HA system, which indicated the generation of HO•. Moreover, detecting the concentration of 7-hydroxycoumarin in the reaction systems through adding coumarin into the reaction solution in advance is an effective method to semi-quantitatively analyze the HO• yield (Louit et al. 2005; Maezono et al. 2011). On account of the high reactivity of HO•, HO• can attack the C-H at the 7-position of coumarin to produce 7-hydroxycoumarin, which is a highly fluorescent compound (Equation (7)). Therefore, coumarin was also employed into the O<sub>2</sub>/Cu/HA system to further identify the generation of HO•. As shown in Figure 3(b), generation of 7-hydroxycoumarin in the O<sub>2</sub>/Cu system was not detected. However, with the addition of 2 mM, the concentration of 7-hydroxycoumarin increased as the reaction progressed in the O<sub>2</sub>/Cu/HA system, and more than 13 μM 7-hydroxycoumarin was generated in the O<sub>2</sub>/Cu/HA system at 120 min. Meanwhile, it should be noted that the concentration of 7-hydroxycoumarin can not completely represent the yield of HO• resulting from the high reaction rate of the reaction between HA and HO• (Equation (6)) and the very short lifetime of HO•.



Based on the strong inhibition of BA degradation by TBA, the specific spectrum of DMPO-OH, and the significant generation of 7-hydroxycoumarin in the O<sub>2</sub>/Cu/HA



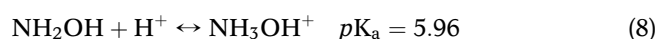
**Figure 3** | Effect of TBA on generation of H<sub>2</sub>O<sub>2</sub> and BA degradation (a), generation of 7-hydroxycoumarin (b), and the intensity of DMPO-OH adduct signal (c) in the O<sub>2</sub>/Cu/HA system. pH = 7 ± 0.2, O<sub>2</sub> flow rate 0.3 L/min, (a) [HA]<sub>0</sub> = 2 mM, [Cu(II)]<sub>0</sub> = 50 μM, [TBA]<sub>0</sub> = 30 mM, [BA]<sub>0</sub> = 10 μM; (b) [HA]<sub>0</sub> = 2 mM, [Cu(II)]<sub>0</sub> = 50 μM, [coumarin]<sub>0</sub> = 2 mM; (c) [HA]<sub>0</sub> = 20 mM, [Cu(II)]<sub>0</sub> = 500 μM, [DMPO]<sub>0</sub> = 100 mM.

system, it can be concluded that the primary reactive oxidant of the O<sub>2</sub>/Cu/HA system is HO•.

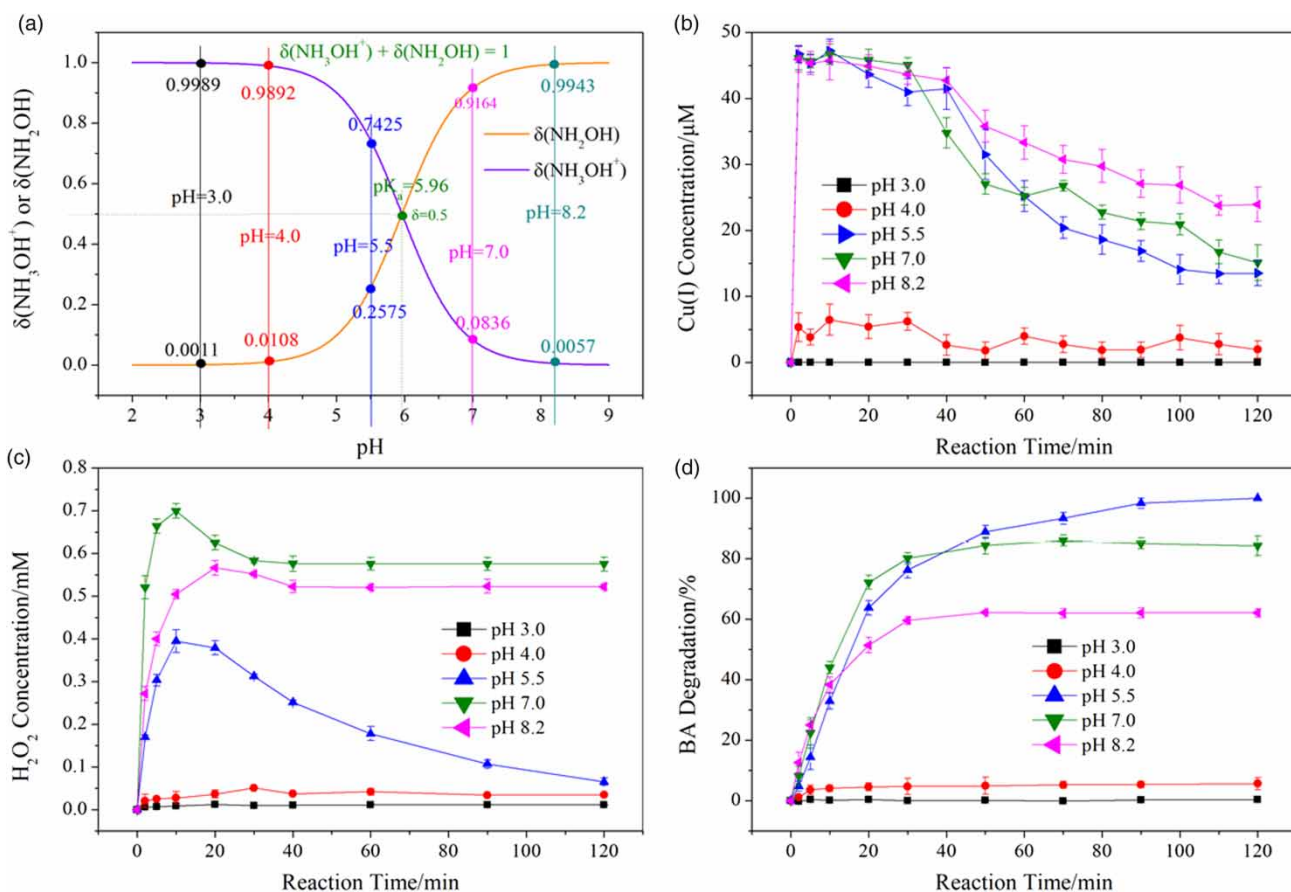
### Effect of pH

To further investigate the mechanism and the effect factor, the effect of pH on the O<sub>2</sub>/Cu/HA system was explored. Figure 4 shows the effect of pH on the distribution fraction ( $\delta$ ) of HA (pK<sub>a</sub> = 5.96) (Robinson & Bower 1961), Cu(I) generation, H<sub>2</sub>O<sub>2</sub> generation, and BA degradation. As can be seen, 99.89% HA was present in the form of NH<sub>3</sub>OH<sup>+</sup> (Equation (8)) and no Cu(I) was generated at pH 3.0. Meanwhile, the generation of H<sub>2</sub>O<sub>2</sub> and BA degradation was not detected. At pH 4.0, the molar ratio of NH<sub>3</sub>OH<sup>+</sup>/HA is 98.90% and little Cu(II) was reduced to Cu(I). The generation of H<sub>2</sub>O<sub>2</sub> is feeble, and only 5.7% BA was degraded in 120 min. Because of the transfer of lone pair electrons in nitrogen to Cu(II), NH<sub>2</sub>OH can reduce Cu(II) into Cu(I). However, due to the protonation of HA at low pH,

protonated HA (NH<sub>3</sub>OH<sup>+</sup>) loses the lone pair electrons which reduces the reduction capacity of HA. Thus, it is really hard for NH<sub>3</sub>OH<sup>+</sup> to reduce Cu(II) into Cu(I). On the contrary, at pH values of 5.5, 7.0, 8.2, the molar ratio of NH<sub>3</sub>OH<sup>+</sup>/HA are 74.25%, 8.36%, and 0.57%, respectively, and Cu(II) was rapidly and almost completely reduced into Cu(I) when the reaction initiated. Moreover, H<sub>2</sub>O<sub>2</sub> was strongly generated, and the peak concentrations of H<sub>2</sub>O<sub>2</sub> were 0.39, 0.70, and 0.57 mM at pH 5.5, 7.0, and 8.2, respectively. After 120 min, 100%, 84%, and 62% BA were degraded in the O<sub>2</sub>/Cu/HA system at pH values of 5.5, 7.0, and 8.2, respectively.



Based on the above results, it can be inferred that NH<sub>3</sub>OH<sup>+</sup> can not reduce Cu(II) into Cu(I), resulting in very little or no H<sub>2</sub>O<sub>2</sub> and HO• generated. Moreover, higher pH may decrease the utilization ratio of HA due to



**Figure 4** | Effect of pH on distribution fraction of HA ( $\delta$ ) (a), generation of Cu(I) (b), generation of H<sub>2</sub>O<sub>2</sub> (c), and BA degradation (d) in the O<sub>2</sub>/Cu/HA system. pH = 3.0, 4.0, 5.5, 7.0, and 8.2, [HA]<sub>0</sub> = 2 mM, [Cu(II)]<sub>0</sub> = 50 μM, O<sub>2</sub> flow rate 0.3 L/min, [BA]<sub>0</sub> = 10 μM.

the higher reaction rate between  $\text{HO}\cdot$  and HA with pH increase (pH 4.0,  $k < 5.0 \times 10^8 \text{ M}^{-1}\text{s}^{-1}$ ; pH 8.0,  $k = 9.5 \times 10^9 \text{ M}^{-1}\text{s}^{-1}$ ) (Buxton *et al.* 1988). More HA was utilized to reduce Cu(II) into Cu(I) at pH 5.5 than that at pH 7.0 and 8.2, resulting in sustained decomposition of  $\text{H}_2\text{O}_2$  to produce  $\text{HO}\cdot$  (Figure 4(c)). For this reason, the BA degradation rate after 120 min decreased with an increase of the pH range from 5.5 to 8.2. Therefore, unprotonated HA is the motive power of the  $\text{O}_2/\text{Cu}/\text{HA}$  system, and pH is a critical factor.

### Effect of HA dosage

Figure 5 shows the effect of HA dosage on the generation of  $\text{H}_2\text{O}_2$  and the degradation of BA in the  $\text{O}_2/\text{Cu}/\text{HA}$  system. The growth in the final  $\text{H}_2\text{O}_2$  yield was observed with the increase of HA dosage in the range of 0.5 mM to 2 mM. However, exceeding this concentration resulted in reduction of the final yield of  $\text{H}_2\text{O}_2$ . When the dosage of HA is under 3 mM, concentration of  $\text{H}_2\text{O}_2$  upgrades significantly rapidly in the first phase (0–10 min), descends gradually from 10 min to 60 min, and remains stable after 60 min. Whereas, when the dosage of HA is greater than 5 mM, the  $\text{H}_2\text{O}_2$  concentration fluctuates firstly then remains stable resulting from the competition between the  $\text{H}_2\text{O}_2$  generation and consumption. A high concentration of HA can accelerate the transformation from Cu(II) to Cu(I) to enhance the formation of  $\text{H}_2\text{O}_2$  (Equations (2) and (3)) (Yuan *et al.* 2012;

Kim *et al.* 2015; Zhou *et al.* 2016a); meanwhile, Cu(I) can consume  $\text{H}_2\text{O}_2$  as a greater activator for  $\text{H}_2\text{O}_2$  (Equation (5)). The final BA degradation in 120 min increased with the growth of HA dosage, whereas excessive HA dosage, such as 8 mM or 10 mM, can inhibit the BA degradation in the initial phase. Resulting from that, the generated  $\text{HO}\cdot$  could be quenched by excess HA (Equation (6)), especially when the dosage of HA is high enough (Buxton *et al.* 1988).

### The role of reducing agents in the $\text{O}_2/\text{Cu}/\text{RAS}$ systems

Based on the results and discussion above, the key step to form reactive oxygen species in the  $\text{O}_2/\text{Cu}/\text{HA}$  system is the generation of Cu(I), which can induce further chain reactions between the copper species and the oxygen-containing substances ( $\text{O}_2$ ,  $\text{H}_2\text{O}_2$ , and  $\text{O}_2\cdot^-$ ). It could be surmised that some other RAs, which can reduce Cu(II) into Cu(I), may take on the role of HA in the  $\text{O}_2/\text{Cu}/\text{HA}$  system. Therefore, the effects of some RAs (NMHA, L-AA, p-HQ, and SN) were investigated in the  $\text{O}_2/\text{Cu}/\text{RAS}$  systems.

Figure 6 shows the generation of Cu(I) and  $\text{H}_2\text{O}_2$ , BA degradation, and 7-hydroxycoumarin generation in the  $\text{O}_2/\text{Cu}/\text{RAS}$  systems after adding NMHA, L-AA, p-HQ and SN, respectively. As shown in Figure 6(a), Cu(I) was produced in the  $\text{O}_2/\text{Cu}/\text{RAS}$  systems with the addition of 1 mM HA, NMHA, or L-AA, and nearly 100% of Cu(II) was rapidly reduced into Cu(I) in the initial phase in these three systems. Corresponding to the generation of Cu(I),

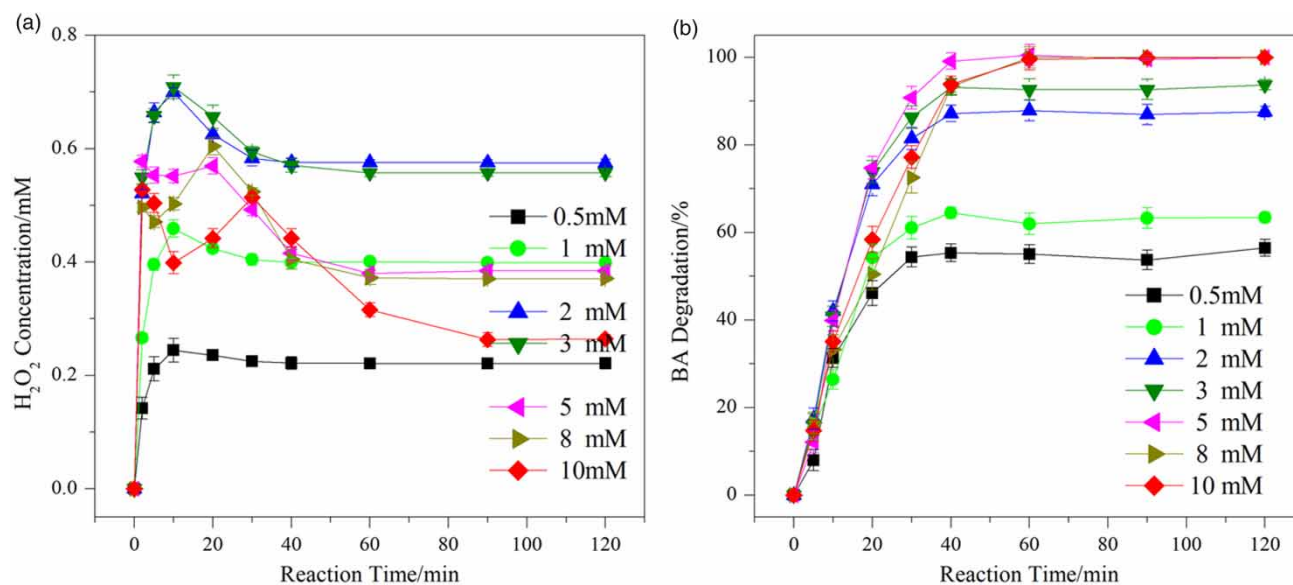
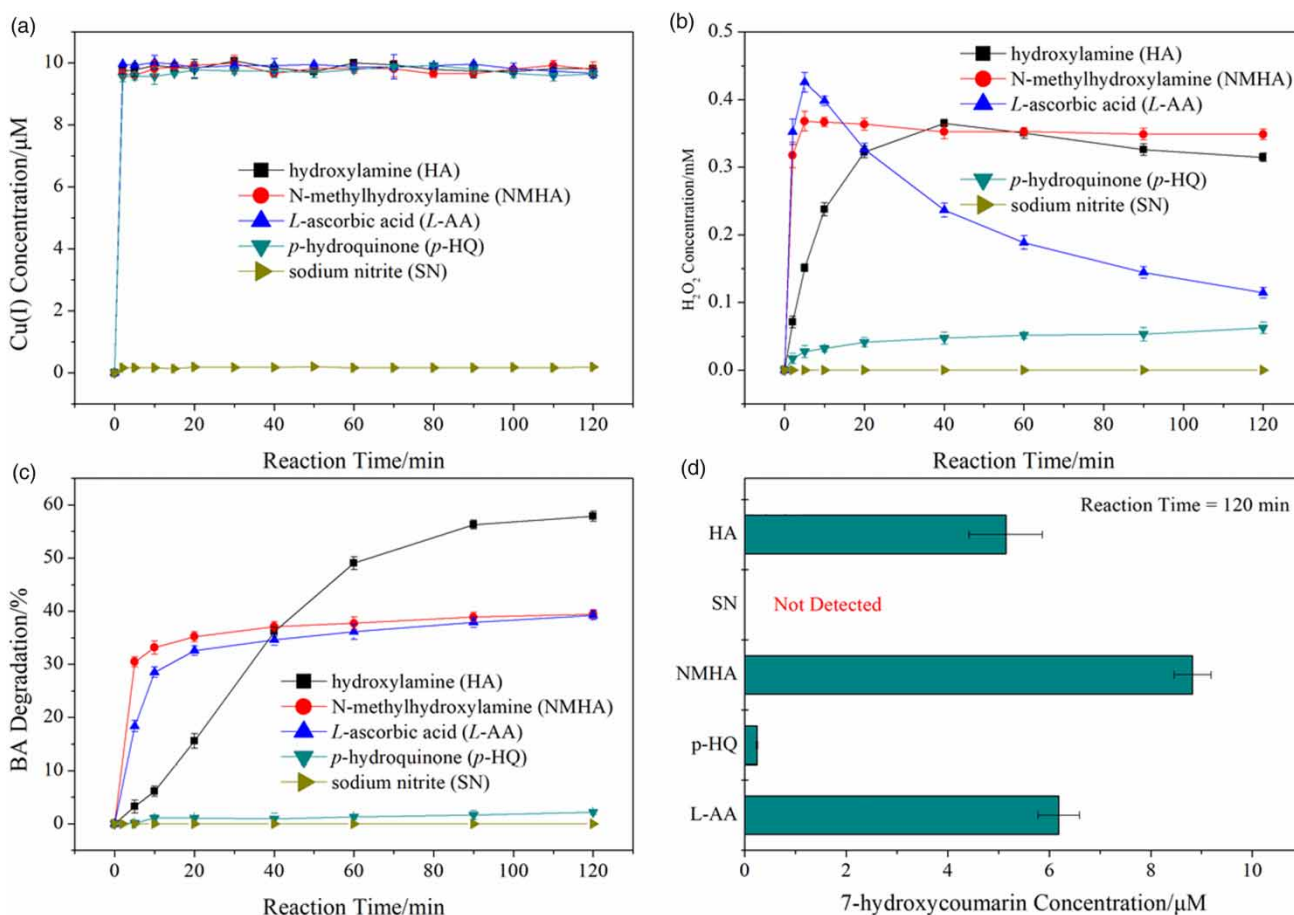


Figure 5 | Effect of HA dosage on generation of  $\text{H}_2\text{O}_2$  (a) and BA degradation (b) in the  $\text{O}_2/\text{Cu}/\text{HA}$  system. pH =  $7 \pm 0.2$ ,  $[\text{HA}]_0 = 0.5, 1, 2, 3, 5, 8, 10 \text{ mM}$ ,  $[\text{Cu(II)}]_0 = 50 \mu\text{M}$ ,  $\text{O}_2$  flow rate 0.3 L/min,  $[\text{BA}]_0 = 10 \mu\text{M}$ .



**Figure 6** | Concentration of Cu(I) (a) and  $H_2O_2$  (b) and BA degradation (c) as a function of reaction time and 7-hydroxycoumarin concentration (reaction time = 120 min) (d) in the  $O_2/Cu/RAS$  systems. pH =  $7 \pm 0.2$ , [RAS (HA, NMHA, L-AA, p-HQ, or SN)]<sub>0</sub> = 1 mM, [Cu(II)]<sub>0</sub> = 10  $\mu M$ ,  $O_2$  flow rate 0.5 L/min, [BA]<sub>0</sub> = 10  $\mu M$  or [coumarin]<sub>0</sub> = 1 mM.

adding these three RAs significantly enhanced the  $H_2O_2$  formation as shown in Figure 6(b), and the  $H_2O_2$  concentrations dramatically increased in the initial phase and then gradually decreased with concentration peaks of 0.36, 0.37, and 0.43 mM, respectively. Furthermore,  $HO\cdot$  was obviously produced in these systems, and the removal rates of BA reached 57.9%, 39.5%, and 39.3%, respectively, and the yields of 7-hydroxycoumarin were respectively 5.1, 8.8, and 6.2  $\mu M$  at 120 min (Figure 6(c) and 6(d)). Moreover, Cu(II) can not be reduced into Cu(I) by SN in the  $O_2/Cu/SN$  system, resulting in the chain reactions between copper species and oxygen-containing substances not being able to be induced. As such, the generation of  $H_2O_2$ , removal of BA, and generation of 7-hydroxycoumarin were also not detected in the  $O_2/Cu/SN$  system.

In addition, almost all the Cu(II) was reduced into Cu(I) by p-HQ in a short time in the initial phase (less than 2 min). According to the previous literature (Yuan *et al.* 2013), p-HQ can be oxidized into p-benzoquinone (p-BQ)

catalyzed by Cu(II)/Cu(I) redox couple in aerobic solution (Figure S1, available with the online version of this paper). Due to p-BQ being an  $O_2\cdot^-$  scavenger (Kishikawa *et al.* 2009; Kawashima *et al.* 2011), the intermediate  $O_2\cdot^-$  in the  $O_2/Cu/p-HQ$  system may be quenched by p-BQ to inhibit the formation of  $H_2O_2$  via Equation (3). Thus, the generation of  $H_2O_2$  in the  $O_2/Cu/p-HQ$  system was relatively lower than that of the  $O_2/Cu/HA$ ,  $O_2/Cu/NMHA$ , and  $O_2/Cu/L-AA$  systems. However, the generation of  $H_2O_2$  was also detected and 0.07 mM  $H_2O_2$  was produced at 120 min in the  $O_2/Cu/p-HQ$  system. Low yield of  $H_2O_2$  inhibited the generation of  $HO\cdot$  via Equation (5); only 3% BA was degraded and 0.25  $\mu M$  7-hydroxycoumarin was generated. Thus, the p-HQ can also play the same role that HA played in the  $O_2/Cu/RAS$  system; notwithstanding, p-BQ can reduce the yield of  $H_2O_2$ .

Based on the results and discussion above, it can be concluded that some other RAs that can reduce Cu(II) into Cu(I) can act as HA in the  $O_2/Cu/HA$  system so as to induce the chain reactions of the copper species to induce



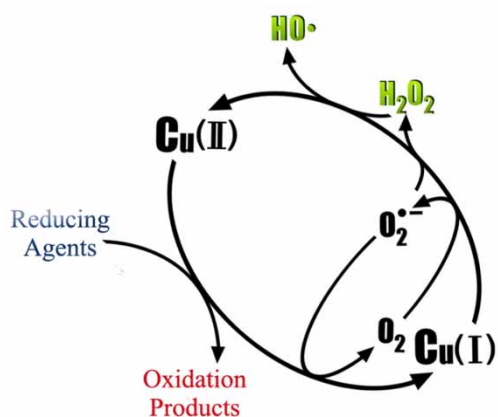


Figure 7 | Proposed mechanism of the  $O_2/Cu/RAs$  systems.

the generation of ROS (e.g.  $O_2^{\cdot-}$ ,  $H_2O_2$ , and  $HO^{\cdot}$ ). The mechanism of the  $O_2/Cu/RAs$  systems was proposed as Figure 7.

## CONCLUSIONS

This study set up the  $O_2/Cu/HA$  system to utilize HA to reduce Cu(II) into Cu(I) so as to initiate the chain reactions of the oxygen species, resulting in the generation of  $O_2^{\cdot-}$ ,  $H_2O_2$ , and  $HO^{\cdot}$  in the aerobic aqueous solutions. The mechanism proposes that unprotonated HA can reduce Cu(II) into intermediate Cu(I) to react with  $O_2$  producing  $O_2^{\cdot-}$ , and  $O_2^{\cdot-}$  can react with Cu(I) to form  $H_2O_2$ . Then,  $H_2O_2$  can be further activated by intermediate Cu(I) via a Fenton-like reaction to produce  $HO^{\cdot}$ , resulting in BA degradation.  $O_2$  is indispensable due to less  $H_2O_2$  being detected and less BA being degraded in the anoxic ( $N_2$ ) aqueous solution. Moreover, pH is a crucial factor in the  $O_2/Cu/HA$  system; resulting from that, the protonated HA ( $NH_3OH^+$ ) can not reduce Cu(II) into Cu(I), and Cu(I), which can initiate the chain reactions, is the pivotal intermediate of the  $O_2/Cu/HA$  system. A high concentration of HA can accelerate the Cu(II)/Cu(I) redox cycle to initiate the  $HO^{\cdot}$  generation, but excessive HA dosage could inhibit the BA degradation in the initial phase. Although a higher Cu(II) dosage could enhance the  $HO^{\cdot}$  generation to degrade BA, a lower dosage of Cu(II) is more beneficial for the accumulation of  $H_2O_2$  (Figure S2, available with the online version of this paper). In addition, some other RAs (e.g. NMHA, L-AA, and *p*-HQ) which can reduce Cu(II) into Cu(I) could act as HA in the  $O_2/Cu/HA$  system so as to induce the chain reactions of the copper species to induce the generation of ROS (e.g.  $O_2^{\cdot-}$ ,  $H_2O_2$ , and  $HO^{\cdot}$ ) with strong oxidation ability.

Therefore, these ROS generated in this new advanced oxidation system ( $O_2/Cu/RAs$  system) can effectively degrade refractory organic contaminants in reclaimed water and wastewater with  $O_2$  as the original oxidant. These systems using RAs and Cu(II) cover wider pH ranges (e.g. 4.2–8.0 for the  $O_2/Cu/HA$  system), comparing to the iron-catalyzed Fenton-like system. However, the concentration and type of copper chelating compounds existing in the reaction solution are potential variable factors (both negative and positive effects) to influence the performance of the  $O_2/Cu/RAs$  system. Structural stable heterogeneous copper catalysts need to be investigated to reduce the environmental risk of homogeneous copper, although the effective dosage of Cu(II) in the  $O_2/Cu/RAs$  system is less than  $15.6 \mu M$  (the maximum level for copper set by the Chinese Ministry of Health). In addition, some other metals with multiple valences which are eco-friendly may be employed to replace copper in the  $O_2/Cu/RAs$  system.

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