

View Article Online View Journal

RSC Advances

This article can be cited before page numbers have been issued, to do this please use: P. Zhou, J. zhang, Y. zhang, Y. Liu, J. Liang, B. Liu and W. Zhang, *RSC Adv.*, 2016, DOI: 10.1039/C6RA02843H.



This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. This Accepted Manuscript will be replaced by the edited, formatted and paginated article as soon as this is available.

You can find more information about *Accepted Manuscripts* in the **Information for Authors**.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard <u>Terms & Conditions</u> and the <u>Ethical guidelines</u> still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.



www.rsc.org/advances

RSC Advances

Generation of Hydrogen Peroxide and Hydroxyl Radical Resulting from Oxygen-Dependent Oxidation of *L*-Ascorbic Acid via Copper-Redox Catalyzing Reactions

Peng Zhou, Jing Zhang*, Yongli Zhang, Ya Liu, Juan Liang, Bei Liu, Wei Zhang College of Architecture & Environment, Sichuan University Chengdu 610065, P. R. China E-mail: zjing428@163.com

Abstract

The generation of hydrogen peroxide (H_2O_2) and hydroxyl radical $(HO\bullet)$ during the oxidation of *L*-ascorbic acid (L-AA) by oxygen with copper as a catalyst was investigated to set up the O₂/Cu/L-AA process, with benzoic acid (BA) as the probe reagent. The generated high concentration of H₂O₂ undergoes an intramolecular two-electron transfer and is



further activated by intermediate cuprous copper [Cu(I)] to induce the product of HO• resulting in significant degradation of BA. Dehydroascorbic acid, 2,3-diketogulonic acid, and L-xylosone were the predominantly detected products of the oxidation of L-AA. However, the H_2O_2 generation and BA degradation were regulated with the variation of pH, which results from the contradiction between protonated L-AA that is difficult to chelate with Cu(II) via electron transfer and hydrion (H⁺) is indispensable for the generation of H₂O₂. Furthermore, the H₂O₂ concentration and BA degradation increased with the increase of L-AA dosage. Trace amounts of Cu(II) is effective to catalyze the oxidation of L-AA, while the H₂O₂ generation and BA degradation increase of the Cu(II) dosage. Due to the formation of Cu(I)-chloride complexes or Cu(II)-chloride complexes, the addition of chloride (Cl⁻) could restrict the generation of H₂O₂ and BA degradation.

Key word: hydroxyl radical; hydrogen peroxide; copper; *L*-ascorbic acid; oxygen

1 Introduction

Copper (Cu) is an essential transition metal that is involved in a variety of physicochemical reactions and physiological processes in natural aquatic systems and is vital to the viability of almost all organisms,^{1,2} principally resulting from the redox transformations between cuprous copper [Cu(I)] and cupric copper [Cu(II)].³ As such, numerous previous studies have focused on oxygen-dependent oxidation of reducing agents catalyzed by redox cycling of copper, and indicated that there are a number of potential important reactions and products.^{4,5} For example, Cu(II) is capable of oxidizing 1,4-hydroquinone, resulting in the formation of the semiquinone anion radical, benzoquinone, and hydrogen peroxide (H₂O₂) through an oxidant-producing copper-redox cycle mechanism.^{6,7} The presence of a suitable ligand can allow Cu(II) to act as an efficient aerobic redox catalyst for 1,4-hydroquinone oxidation.⁸ Moreover, copper observably catalyzes the oxidation of 2,3-Dihydroxybenzoic acid, which follows the generation of H₂O₂ and hydroxyl radical (HO•).⁹ What the processes have in common is that Cu(I) is widely considered as a vitally important intermediate.

Cu(I) is also considered to be an important scavenger of O_2 .^{10,11} Redox reactions of Cu(I) could result in generation of reactive oxygen species, such as superoxide radical (O_2^{-}) and H_2O_2 , that may subsequently induce a series of promoted reactions with other constituents in natural waters.^{12,13} A significant production of HO•,¹⁴ could be generated from the further reaction between Cu(I) with H_2O_2 . Although Cu(I) is an excellent activator for H_2O_2 to induce the product of HO•, Cu(I) was seldom investigated to activate H_2O_2 owing to its instability and difficulty in dissolving.¹⁵ As commonly seen, the capacity of the reducing agent to reduce Cu(II) into Cu(I) is a decisive prerequisite to induce chain reactions resulting in the generation of reactive species in the copper catalyzed reducing agents oxidation processes.

L-ascorbic acid (L-AA, vitamin C), which has been proved to be an important antioxidant in both plant and animal tissues by preventing oxidative-induced cellular damage, is a water-soluble vitamin that is a necessary component for human health.¹⁶ It is able to reduce Cu(II) into Cu(I), and further induce the generation of H_2O_2 .^{17,18} Meanwhile, L-AA may overcome the drawback of Cu(I) through reducing Cu(II) into Cu(I) to immediately activate intermediate H_2O_2 to product HO•. However, the concomitant formation of H_2O_2 and HO•, and consumption of oxygen are not thoroughly studied in water treatment. Especially, the generation and production of HO• received little attention in the previous literatures, although HO• was recognized as an iconic intermediate

in advanced oxidation processes (AOPs). Other than traditional AOPs, the generation of HO• through oxidation of L-AA catalyzed by copper occurs without any dosage of conventional oxidizers, such as H_2O_2 , ozone, persulfate, and peroxymonosulfate, but it is induced from a most frequent oxidizer O_2 , which was seldom reported in the previous literatures.

This study aims to investigate the generation of H_2O_2 and degradation of BA in the $O_2/Cu/L$ -AA process, specifically focused on the mechanism of the generation of H_2O_2 and HO• during the L-AA oxidation catalyzed by copper, while benzoic acid (BA) was selected as the model compound to indirectly reveal the production of HO•.¹⁹ The products of L-AA in the $O_2/Cu/L$ -AA process were examined with the gas chromatography/mass spectroscopy (GC/MS) technique. Moreover, the effect of pH, L-AA dosage and Cu(II) dosage in the $O_2/Cu/L$ -AA process were investigated. The chloride ion was introduced into the $O_2/Cu/L$ -AA process to investigate the effect of copper-chloride complexes on the $O_2/Cu/L$ -AA process.

2 Materials and methods

2.1 Materials

Benzoic acid (BA, \geq 99.5%) and copper sulfate pentahydrate (CuSO₄·5H₂O, \geq 99.0%) are of analytic purity and were supplied by Sigma-Aldrich. *L*-ascorbic acid (L-AA, \geq 99.7%), hydrogen peroxide (H₂O₂, 30%), *tert*-butyl alcohol (TBA, \geq 99.5%), phosphoric acid, monosodium phosphate (\geq 99.0%), sodium hydrogen phosphate (\geq 99.0%), sodium thiosulfate (\geq 99.0%), sodium chloride (\geq 99.5%), and potassium titanium oxalate (\geq 98.5%) are of analytic purity and were purchased from Sinopharm Chemical Reagent Co., Ltd. Methanol (\geq 99.9%), phosphoric acid, and dichloromethane (\geq 99.9%), which were purchased from Sigma-Aldrich, were of HPLC grade. Pure oxygen (O₂, \geq 99.2%) was stored in a special high-pressure gas cylinder.

2.2 Procedures

Most of the experiments were carried out at 25 ± 1 °C with a 500 mL beaker by heating in water bath under constant feeding of pure O₂ through an aerator in Milli-Q water (18.25 M Ω ·cm). In order to investigate the consumption rate of oxygen in the processes, part of experiments were carried out in a 500 mL sealed florence flask under constant stirring with a PTFE-coated magnetic stirrer. Benzoic acid and Cu(II) (CuSO₄) with desired concentrations were spiked in 500 mL phosphoric acid-phosphate buffer. Each run was initiated by adding the desired dosage of fresh L-AA. The pH changed less than ± 0.2 during the process. Samples were respectively withdrawn

at set intervals and quenched by sodium thiosulfate (for BA) or chelated by potassium titanium oxalate (for H_2O_2) before analysis. The quenching experiments employed *tert*-butyl alcohol as quencher, which was introduced in excess immediately after the addition of L-AA. The chloride ion was introduced into the experiments to investigate the effect of chelated Cu(II) on the $O_2/Cu/L$ -AA process.

2.3 Analysis

The concentration of BA was analyzed on HLPC (Waters, e2695), equipped with the reverse-phase C18 column (4.6×150 mm). The binary phase consisted of (A) water with 0.1% H₃PO₄ and (B) methanol, and the eluent was A and B (58:42, v/v) with a flow rate of 1.0 mL/min. Detection was performed using a 2489 λ UV absorbance detector set at 227 nm for BA.

The products of L-AA oxidation were examined with the gas chromatography/mass spectroscopy (GC/MS) technique, operating on a QP2010Plus GC/MS. Prior to GC/MS determination, a 20 mL sample was extracted using 10 mL dichloromethane three times under acidic (pH \approx 2.0), neutral (pH \approx 7.0), and alkaline (pH \approx 12.0) conditions, respectively. The three extracted layers were mixed, dehydrated and concentrated to 5 mL under nitrogen atmosphere. The prepared dichloromethane solution was filtered by the 0.22 µm polytetrafluoroethylene membranes and stocked in amber bottles before analysis.

Furthermore, the pH was measured by a pH meter (PHB-4). After chelated by potassium titanium oxalate, the H_2O_2 concentrations were measured on UV-vis spectrometer (MAPADA, UV-1800) at 400 nm using a 1cm quartz cuvette, and the concentration of dissolved oxygen (DO) was measured by a dissolved oxygen meter (JPB-607A). Every experiment was carried out three times and the standard deviation obtained was less than 2.0%.

3 Results and discussion

3.1 Generation of H₂O₂ and HO• in the O₂/Cu/L-AA process.

The generation of H_2O_2 during the oxidation of 0.8 mM of L-AA catalyzed by copper was investigated, with the results shown in Figure 1. Astoundingly, the highest concentration of H_2O_2 unexpectedly went up to 0.48 mM. Simultaneously, more than 40% of BA was degraded in the $O_2/Cu/L$ -AA process. It is reasonable to presume that BA was degraded by HO• which could be generated for the copper catalyst-mediated decomposition of some reducing agents.^{6,18}

To identify the contribution of HO• on the degradation of BA, TBA was introduced into the

 $O_2/Cu/L$ -AA process, owing to its high reaction rate with HO• ($k = 6.0 \times 10^8 \text{ M}^{-1} \text{s}^{-1}$).²⁰ As shown in Figure 1, in the absence and presence of TBA, the variations of H₂O₂ concentration over time are similar. However, the addition of 25 mM TBA (2500 times of the initial BA concentration) almost completely inhibited the BA degradation. Thus, it could be concluded that the primary reactive oxidant was HO• in the O₂/Cu/L-AA process.

3.2 Pathway of the Generation of H₂O₂ and HO•

L-AA is a binary acid with a bifunctional ene-diol group built into a heterocyclic lactone ring, as indicated by Formula **1** in Scheme 1. Due to resonance stabilization between the oxygens at the 1and 3-positions, the high acidity of the 3-hydroxyl is easily ionized and the undissociated hydroxyl group at 2-position of the monoanion may be hydrogen bonded to the adjoining negatively charged oxygen at the 1-positions, as indicated by Formula **2**. In addition, L-AA is a strong two-electron reducing agent that is readily oxidized in one-electron steps by metal ions and metal complexes in their higher valence states.¹⁸

By using copper as a catalyst, the oxidation of L-AA was involved in a classical chain reactions, and an inner sphere mechanism is illustrated by Scheme 1.^{17,18} The first step of the oxidation of L-AA is the interaction between Cu(II) and Formula **2** to form a monoprotonated Cu(II) complex, indicated by Formula **3**. The monoprotonated Cu(II) complex is an ephemeral intermediate, and rapidly undergoes an intramolecular one-electron transfer to give an unprotonated Cu(II) complex (Formula **4**) or another monoprotonated Cu(II) complex (Formula **5**). Formula **5** in turn undergoes a second intramolecular electron transfer to produce dehydroascorbic acid (D-AA, Formula **6**), the final product of the oxidation of L-AA. Moreover, Cu(I), a strong reducing agent, and O_2^{--} are generated with the intramolecular electron transfer. Nevertheless, under the attack from hydrion (H⁺), Formula **4** finally decompose into D-AA, Cu(II), and H₂O₂.

Accidentally, Cu(I) and H₂O₂ form a Fenton-like system in the O₂/Cu/L-AA process. Cu(I) is actually a great activator for H₂O₂ to induce the generation of HO•, but limited by its instability and difficulty in dissolving. However, as intermediates, Cu(I) and H₂O₂ could be in full use to produce HO• in the O₂/Cu/L-AA process, especially when the yield of H₂O₂ is high. The main reactions considered in the O₂/Cu/L-AA process is presented as follow Eqs. (1)-(5), due to low rate constant, many of these possible reactions in this system were excluded from the main mechanism.^{6,10,12} Furthermore, products Cu(I) and H₂O₂ contribute to enhance the generation of

HO•, via Eq. (2) and Eq. (3) respectively, and it could infer that the consumption of H_2O_2 via Eq. (4) and Eq. (5), which explained why the concentration of H_2O_2 increased firstly then decreased latter in the $O_2/Cu/L$ -AA process over time as shown in Figure 1.

$$Cu(I) + O_2 \longrightarrow Cu(II) + O_2^{\bullet-} \qquad 0.48 \text{ M}^{-1} \text{s}^{-1} \qquad (1)$$

$$Cu(II) + O_2^{\bullet-} \to Cu(I) + O_2$$
 $6.6 \times 10^8 \,\mathrm{M}^{-1} \mathrm{s}^{-1}$ (2)

$$Cu(I) + O_2^{\bullet-} \xrightarrow{2H^+} Cu(II) + H_2O_2$$
 $2.0 \times 10^9 \,\mathrm{M}^{-1}\mathrm{s}^{-1}$ (3)

$$Cu(I) + H_2 O_2 \xrightarrow{2H^+} Cu(II) + HO^{\bullet} + OH^- \qquad 4.7 \times 10^3 \text{ M}^{-1} \text{s}^{-1}$$
(4)

$$Cu(II) + H_2 O_2 \xrightarrow{-2H^+} Cu(I) + O_2^{\bullet-}$$
 460 M⁻¹s⁻¹ (5)

3.3 Products of L-AA

In order to investigate the further oxidation of L-AA catalyzed by copper, the oxidation products of L-AA were identified by GC/MS analysis. As shown in Figure 2, D-AA (peak area, 31.69%) was the predominantly detected compound in the O₂/Cu/L-AA process, which conforms to the two-electron transfer oxidation of L-AA as seen in scheme 1. Moreover, 2,3-diketogulonic acid (DKGA, peak area, 16.33%) and L-xylosone (LX, peak area, 9.44%) were found to be the other important constituents. In previous reports,^{21,22} L-AA is firstly oxidized to D-AA and then form DKGA, after which the decarboxylation of DKGA forms LX. It could be inferred that the further oxidation of L-AA by copper as a catalyst may follow the same pathway. Furthermore, several obvious peaks eluted in GC/MS chromatogram could not be identified properly, due to lack of authentic standards. However, the presence of several of unidentified compounds probably formed through the compounds, such as L-AA, D-AA, DKGA, LX, and so on, attacked by HO• or other reactive oxidants in the O₂/Cu/L-AA process.

3.4 Effect of pH

To further investigate the mechanism of the $O_2/Cu/L$ -AA process, the effect of pH on the generation of H_2O_2 and the degradation of BA was studied. As shown in Figure 3a, in the pH range from 2.8 to 8.3, the generation of H_2O_2 was obvious, and the H_2O_2 concentrations increased and then declined over time in all processes. In addition, the peak of H_2O_2 concentration appeared later with the pH decreased, and the generation of H_2O_2 is strongly enhanced at pH 4.5. As shown in Scheme 1, the generation of H_2O_2 with pH may be enhanced if the concentration of L-AA is partly replaced by that of the monoanion (Formula **2**). Firstly, the formation of the intermediate

monoprotonated (Formula 3), which is the reactive species in $O_2/Cu/L$ -AA process, is scarcely possible when the 3-hydroxyl is not ionized. However, without the attack by H⁺, it is difficult for Formula 3 and Formula 4 to form D-AA and H₂O₂ through electron transfer. It should be noted that about 68% of L-AA here existed in the form of Formula 2 with pK_{a1} = 4.10.²³ Thus, the generation of H₂O₂ was regulated by H⁺ with the pH decrease, although the decrease of pH could enhance the formation of Formula 3.

As shown in Figure 3b, increase of BA degradation was observed with the increase of pH range from 2.8 to 4.5, then the increase of pH resulted in a decrease of BA removal. Obviously, the degradation of BA with the variation of pH greatly conforms to the generation of H₂O₂, resulting from that the elevated levels of H₂O₂ could accelerate the generation of HO• via Eq. (4) to degrade BA. The generation of O₂⁻⁻ and Cu(I) were regulated by H⁺, which is similar to the effect of H⁺ on H₂O₂ generation; it was another way for pH to impact on BA degradation. Moreover, the degradation of L-AA by HO• is not ignorable, and the rate constant between L-AA and HO• increased with the increase of pH.²⁰ Therefore, more L-AA would exist with the form of Formula **2** and less H⁺ was involved in the generation of H₂O₂ and Cu(I) with the increase of pH, which could be the major causes to the variation of H₂O₂ and Cu(I) with the increase of pH.

3.5 Effect of L-AA concentration.

To further investigate the role of L-AA, the effect of L-AA concentration on H₂O₂ generation and BA degradation in the O₂/Cu/L-AA process was studied. As shown in Figure 4, with the increase of pH in the range of 0.1 to 3.0 mM, the generation of H₂O₂ was strongly enhanced, while, resulting in the increase of the degradation of BA. Moreover, the degradation of BA was inhibited in the initial phase of the O₂/Cu/L-AA process, especially when the L-AA dosage is high. It should be noted that the degradation of BA was regulated by L-AA. Although the increase of L-AA concentration could accelerate the generation of H₂O₂ and Cu(I) to induce the generation of HO•, large amount of HO• could be quenched by excess L-AA ($k = 1.1 \times 10^{10} \text{ M}^{-1} \text{s}^{-1}$)²⁰ in the initial phase. However, due to the continual feeding of the oxygen into the solution with the oxidation of L-AA, BA could be continually degraded until the L-AA was completely oxidized to terminate the process. Hence, in improving the degradation efficiency of probe compounds at the highest extent and reducing the cost, a proper dosage of L-AA should be selected.

3.6 Effect of Cu(II)

The process which uses copper as a catalyst described above, accomplishing two-electron oxidation of L-AA, may be employed in catalytic process in which the Cu(II) is only a minor constituent.¹⁷ As shown in Figure 5a, the generation of H₂O₂ was significant when the dosage of Cu(II) is only 2µM, and the H₂O₂ concentration decreased with the increase of Cu(II) dosage in the O₂/Cu/L-AA process. The higher dosage of Cu(II) could accelerate the oxidation of L-AA to produce H_2O_2 , thus, the generation of H_2O_2 was accelerated in the initial phase with the increase of Cu(II) dosage as shown in Figure 5a. However, the increase of the Cu(II) dosage could also enhance the generation of Cu(I), which could accelerate the decomposition of H_2O_2 to product HO• via Eq. (4). Precisely because of the enhanced HO• generation, the degradation of BA was increased with the increase of the Cu(II) dosage, as shown in Figure 5b.

In natural waters, the variety of potential copper-binding ligands is large, and the determination of the actual Cu(I) and Cu(II) species present is difficult and the elucidation of constant rates for reaction of these species is essentially impossible. However, the effect of ligand of copper species on the O₂/Cu/L-AA process is worth investigating. As shown in Figure 6a, the generation of H_2O_2 was enhanced with the dosage of Cl⁻ in the range of 1 to 10 mM. The addition of Cl⁻ at these dosages could almost not form Cu(II)-chloride complexes with Cu(II) resulting from the relatively low affinity of Cl⁻ for Cu(II).⁸ In addition, these dosages of Cl⁻ had minimal effect on the oxygen consumption rates in the $O_2/Cu/L$ -AA process as shown in Tab. 1. Therefore, it could be concluded that the addition of Cl⁻ in the range of 1 to 10 mM has less impact on the consumption of oxygen and the generation of H_2O_2 through electron transfer catalyzed by copper. However, Cu(I)-chloride complexes (CuCl, CuCl₂, and CuCl₃) are expected as the predominant Cu(I) species due to the higher dosages of Cl⁻ in this work and the large stability constants of these complexes.¹² The constant rates of the reaction between H₂O₂ with Cu(I) are inhibited when the Cu(I) species are in the form $CuCl_2$, $CuCl_2^-$, and $CuCl_3^-$, which could inhibit the generation of HO. Although HO• can reacts rapidly with Cl⁻, the reaction forms ClOH⁻• reversibly, and the formation of Cl• generally is only significant at low pH as Eq. (6) and Eq. (7).²⁴ It conforms that the addition of Cl⁻ obviously inhibited the degradation of BA as shown in Figure 6b.

 $HO^{\bullet} + Cl^{-} \leftrightarrow ClOH^{\bullet-}$ $k_{for} = 4.3 \times 10^9 \,\mathrm{M}^{-1} \mathrm{s}^{-1}$ $k_{rev} = 6.1 \times 10^9 \,\mathrm{s}^{-1}$ (6) RSC Advances Accepted Manuscript

$$ClOH^{\bullet-} + H^+ \leftrightarrow Cl^{\bullet} + H_2O$$
 $k_{\rm for} = 2.1 \times 10^{10} \,{\rm M}^{-1}{\rm s}^{-1}$ (7)

Furthermore, when the dosage of Cl⁻ ranges from 30 to 500 mM, the Cu(II) species mainly exist in the form of Cu(II)-chloride complexes $(CuCl^+ \text{ and } CuCl_2)^8$ and the oxygen consumption rate was significantly decreased in the O₂/Cu/L-AA process as shown in Tab. 1. It could be concluded that the high addition of Cl⁻ may inhibit the electron transfer of oxidation of Formula **2** catalyzed by Cu(II) to form Formula **3** resulting in the generation of H₂O₂, which conforms to the generation of H₂O₂ as shown in Figure 6a. It is clear from these results that at proper concentration, Cl⁻ is an effective coordinating agent for copper redox catalysis for generation of H₂O₂, but not for HO•.

4. Conclusions

The study introduced an ignored phenomenon that the strong generation of H_2O_2 and HO_{\bullet} during the oxygen-dependent oxidation of L-AA catalyzed by Cu(II)/Cu(I) redox couple. Undergoing an intramolecular two-electron transfer, high concentration of H_2O_2 was generated and further activated by intermediate Cu(I) to induce the product of HO_• resulting in significant degradation of BA in the O₂/Cu/L-AA process. Dehydroascorbic acid (D-AA), 2,3-diketogulonic acid (DKGA), and L-xylosone (LX) were the predominantly detected products of the oxidation of L-AA based on GC/MS technique.

Moreover, pH and the form of Cu(II) species and Cu(I) species are important influencing factors of the H_2O_2 generation and BA degradation via regulating the election transfer process. Trace amounts of Cu(II) is effective to catalyze the oxidation of L-AA. Meanwhile the H_2O_2 generation and BA degradation increased with the increased dosage of Cu(II) and L-AA. The presence of chloride with low dosage could enhance the concentration of H_2O_2 through forming Cu(I)-chloride complexes to inhibit the activation of H_2O_2 by Cu(I) species which decreases the BA degradation efficiency, and the high dosage of chloride could simultaneously decrease the concentration of H_2O_2 and the degradation efficiency of BA through forming Cu(II)-chloride complexes to inhibit electron transfer.

In the O₂/Cu/L-AA process, L-AA is the driving force resulting from that L-AA can reducing Cu(II) into Cu(I) which is the key intermediate to induce the chain reactions to produce reactive oxygen species. Based on the role of L-AA in the O₂/Cu/L-AA process, it could be inferred that

other reducing agents which can reduce Cu(II) into Cu(I) may act similar to L-AA in the $O_2/Cu/L$ -AA process. Nevertheless, it should be noted the $O_2/Cu/L$ -AA process is far from practical. Our work just introduced an interesting phenomenon and proposed a preliminary interpretation that the oxidation of L-AA by copper catalyzed could induce the production of H₂O₂ and HO•.

Acknowledgements

Appreciation and acknowledgment are given to the National Natural Science Foundation of China (No. 51508353), the National Natural Science Foundation of China (No. 51408349), the National Natural Science Foundation of China (No. 51008052) and the Program for New Century Excellent Talents in University (NCET-11-0082).

References

- 1. A. G. Lewis and W. R. Cave, Oceanogr. Mar. Biol, 1982, 20, 471-695.
- 2. B. R. Stern, J. Toxicol. Env. Heal. A, 2010, 73, 114-127.
- 3. J. A. Mobley, A. S. Bhat and R. W. Brueggemeier, Chem. Res. Toxicol, 1999, 12, 270-277.
- 4. Y. B. Li and M. A. Trush, Carcinogenesis, 1993, 14, 1303-1311.
- 5. M. R. Maurya and S. Sikarwar, J. Mol. Catal. A: Chem, 2007, 263, 175-185.
- 6. X. Yuan, A. N. Pham, C. J. Miller and T. D. Waite, Environ. Sci. Technol, 2013, 47, 8355-8364.
- 7. Y. B. Li and M. A. Trush, Arch. Biochem. Biophys, 1993, 300, 346-355.
- 8. S. Mandal, N. H. Kazmi and L. M. Sayre, Arch. Biochem. Biophys, 2005, 435, 21-31.
- 9. R. Liu, B. Goodell, J. Jellison and A. Amirbahman, Environ. Sci. Technol, 2005, 39, 175-180.

10. M. Gonzalez-Davila, J. M. Santana-Casiano, A. G. Gonzalez, N. Perez and F. J. Millero, *Mar: Chem*, 2009, **115**, 118-124.

- 11. V. K. Sharma and F. J. Millero, Environ. Sci. Technol, 1988, 22, 768-771.
- 12. X. Yuan, A. N. Pham, G. W. Xing, A. L. Rose and T. D. Waite, *Environ. Sci. Technol*, 2012, 46, 1527-1535.
- 13. B. M. Voelker, D. L. Sedlak and O. C. Zafiriou, Environ. Sci. Technol, 2000, 34, 1036-1042.
- 14. Y. B. Li, P. Kuppusamy, J. L. Zweier and M. A. Trush, Chem. Biol. Interact, 1995, 94, 101-120.
- 15. J. W. Moffett and R. G. Zika, Environ. Sci. Technol, 1987, 21, 804-810.

16. M. W. Davey, M. Van Montagu, D. Inze, M. Sanmartin, A. Kanellis, N. Smirnoff, I. J. J. Benzie, J.

- J. Strain, D. Favell and J. Fletcher, J. Sci. Food Agric, 2000, 80, 825-860.
- 17. S. Uluata, D. J. McClements and E. A. Decker, J. Agric. Food Chem, 2015, 63, 1819-1824.
- 18. A. E. Martell, Adv. Chem. Ser, 1982, 200, 153-178.
- 19. X. L. Zhou and K. Mopper, Mar. Chem, 1990, 30, 71-88.
- 20. G. V. Buxton, C. L. Greenstock, W. P. Helman and A. B. Ross, *J. Phys. Chem. Ref. Data*, 1988, 17, 513-886.
- 21. J. P. Yuan and F. Chen, J. Agric. Food Chem, 1998, 46, 5078-5082.
- 22. R. V. Tikekar, R. C. Anantheswaran, R. J. Elias and L. F. LaBorde, *J. Agric. Food Chem*, 2011, **59**, 8244-8248.
- 23. F. Malem and D. Mandler, Anal. Chem, 1993, 65, 37-41.
- 24. Y. Yang, J. J. Pignatello, J. Ma and W. A. Mitch, Environ. Sci. Technol, 2014, 48, 2344-2351.

[CuSO ₄], μM	[Cl ⁻], mM	Initial rate, µM/min
10	0	126.6±0.4
10	1	127.2±0.8
10	3	126.8±0.3
10	5	131.3±1.2
10	10	128.8±1.3
10	30	86.3±0.5
10	100	19.1±0.2
10	500	7.5±0.1

Table 1. Effect of chloridion on the initial oxygen consumption rate in the O₂/Cu/L-AA process.

Conditions: $[L-AA]_0 = 0.5 \text{ mM}$, $[Cu(II)]_0 = 10 \text{ }\mu\text{M}$, $[BA]_0 = 10 \text{ }\mu\text{M}$, $[CI]_0 = 0, 1, 3, 5, 10, 30, 100$, and 500 mM, $[O_2]_0 = 7.24 \pm 0.02 \text{ mg/L}$, $pH = 7 \pm 0.2$, 25 °C. The experiments were carried out at a 500 mL sealed florence flask.

RSC Advances Accepted Manuscript

Published on 31 March 2016. Downloaded by Middle East Technical University (Orta Dogu Teknik U) on 04/04/2016 09:12:55.



Scheme 1. Generation of hydrogen peroxide and hydroxyl radical resulting from oxygen-dependent oxidation of *L*-ascorbic acid via copper-redox catalyzing Reactions.

RSC Advances Accepted Manuscript



Figure 1. Effect of TBA on H_2O_2 generation and BA degradation in the $O_2/Cu/L$ -AA process. Conditions: $[L-AA]_0 = 0.8 \text{ mM}$, $[Cu(II)]_0 = 10 \mu \text{M}$, $[BA]_0 = 10 \mu \text{M}$, $[TBA]_0 = 25 \text{ mM}$, O_2 flow rate = 0.4 L/min, pH = 7 ± 0.2, 25 °C.



Figure 2. The GC/MS chromatogram of the products of the oxidation of L-AA in the $O_2/Cu/L$ -AA process.

Conditions: $[L-AA]_0 = 1.0$ mM, $[Cu(II)]_0 = 10 \mu$ M, O₂ flow rate = 0.5 L/min, pH = 7 ± 0.2, 25 °C, reaction time = 150 min.



Figure 3. Effect of pH on H₂O₂ generation (a) and BA degradation (b) in the O₂/Cu/L-AA process. Conditions: $[L-AA]_0 = 1 \text{ mM}$, $[Cu(II)]_0 = 10 \text{ }\mu\text{M}$, $[BA]_0 = 10 \text{ }\mu\text{M}$, O₂ flow rate = 0.4 L/min, pH = 2.8, 4.5, 5.9, 7.0, and 8.3, 25 °C.



Figure 4. Effect of L-AA concentration on H_2O_2 generation (a) and BA degradation (b) in the $O_2/Cu/L$ -AA process.

Conditions: $[L-AA]_0 = 0.1, 0.3, 1.0, 2.0, and 3.0 \text{ mM}, [Cu(II)]_0 = 10 \text{ }\mu\text{M}, [BA]_0 = 10 \text{ }\mu\text{M}, O_2 \text{ flow rate} = 0.4 \text{ L/min}, \text{ pH} = 7 \pm 0.2, 25 \text{ }^{\circ}\text{C}.$



Figure 5. Effect of Cu(II) dosage on H_2O_2 generation (a) and BA degradation (b) in the $O_2/Cu/L$ -AA process.

Conditions: $[L-AA]_0 = 1.0$ mM, $[Cu(II)]_0 = 2, 5, 10, 30, 50, and 100 \mu$ M, $[BA]_0 = 10 \mu$ M, O_2 flow rate = 0.4 L/min, pH = 7 ± 0.2, 25 °C.



Figure 6. Effect of chloridion on H_2O_2 generation (a) and BA degradation (b) in the $O_2/Cu/L$ -AA process.

Conditions: $[L-AA]_0 = 0.5 \text{ mM}$, $[Cu(II)]_0 = 10 \text{ }\mu\text{M}$, $[BA]_0 = 10 \text{ }\mu\text{M}$, $[CI^-]_0 = 0, 1, 3, 5, 10, 30, 100$, and 500 mM, O₂ flow rate = 0.5 L/min, pH = 7 ± 0.2, 25 °C.