Distinct effects of copper on the degradation of β-lactam antibiotics in fulvic acid solutions during light and dark cycle

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Abstract

This study revealed the dual roles of Cu(II) on the β-lactam antibiotics degradation in Suwannee River fulvic acid (SRFA) solution during day and night cycle. Amoxicillin (AMX) and ampicillin (AMP) were transformed after secondary treatment [8]. AMX and AMP were selected as the representative β-lactam antibiotics. Cu(II) played a key role in the dark degradation of AMX and AMP via catalytic hydrolysis and oxidation. However, Cu(II) mainly exhibited an inhibitory effect on SRFA-involved photochemical degradation of AMX and AMP. In the presence of 500 nM of Cu(II), the degradation rate of AMX and AMP in the light condition were around 5 times higher than that in the dark condition, suggesting the photodegradation of β-lactam antibiotics was much more pronounced than catalyzed hydrolysis and oxidation. The triplet excited state of SRFA (3SRFA*) primarily contributed to AMX and AMP photodegradation. Hydroxyl radicals (·OH) and singlet oxygen (1O2) exhibited limit impacts. The redox cycle of Cu(II)/Cu(I) restricted the electron transfer pathway of 3SRFA* with AMX and AMP. During the day and night cycles for 48 h, Cu(II) served as a stronger inhibitor rather than a promotor. These findings highlight the interactions between Cu(II) and SRFA are distinct under day and night conditions, which could further affect the fate of β-lactam antibiotics in natural environments.

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1. Introduction

β-lactam antibiotics are broad-spectrum antibiotics being widely used as human and veterinary medicine [1,2]. They are among the largest category of antibiotic consumption in the United States, Turkey, India, and China [3]. After being taken by human or livestock, a large portion of β-lactam antibiotics were excreted without undergoing metabolism and flowed into a municipal wastewater treatment system or directly discharged into environment [4,5], However, β-lactam antibiotics cannot be completely eliminated when entering in wastewater treatment plant [6,7]. For example, around 70–81% of amoxicillin (AMX) and ampicillin (AMP) were transformed after secondary treatment [8]. AMX and AMP were observed with concentrations ranging from ng L−1 to μg L−1 in surface waters [9,10]. The presence of β-lactam antibiotics in the aqueous environment may pose a threat to eco-system, such as presenting chronic toxicity to aqueous animals and human [5,11] and promoting the growth of resistant bacteria and the spread of resistant genes [2,12].

The β-lactam ring is liable to attack by various nucleophiles, such as acids, bases, metal ions, and oxidizing agents [6]. The two important degradation pathways for β-lactam antibiotics in the aqueous environment are hydrolysis and photolysis. The hydrolysis of β-lactam antibiotics has been reported to be catalyzed by the presence of various metal ions, such as Fe(III), Mn(II) and Cu(II) [13–15]. Cu(II) has distinct features among these metal ions, as it plays dual roles in the degradation of β-lactam antibiotics [16]. Besides the Cu(II) catalytic hydrolysis of the β-lactam ring, the redox cycle of Cu(II) and Cu(I) also led to the direct oxidation of the phenyglycine primary amine group [17]. Therefore, AMX and AMP are susceptible to Cu(II) catalyzed hydrolysis and oxidation. Forming a Cu(II)-lactam complex is the initial important step to initiate these reactions [16,17].

Degradation of β-lactam antibiotics mediated by sunlight includes direct and indirect photolysis. For antibiotics (including AMX and AMP) without absorption spectra overlapped with solar spectrum, indirect photolysis induced by the dissolved organic
matter (DOM) can promote their photodegradation as a photosensitizer [18]. DOM served as a photosensitizer due to the generation of triplet excited state of dissolved organic matter (DOM*), hydroxyl radicals (OH), singlet oxygen (1O2) and hydrogen peroxyde (H2O2) [19–21]. The indirect photodegradation of AMX has been reported to be dominantly contributed by DOM* [22]. In DOM-rich surface water, reaction with DOM* via electron transfer pathway was dominant leading to the photodegradation of a variety of β-lactam antibiotics [23].

Metal ions and DOM generally coexist in surface water. Although the role of either metal ions or DOM on β-lactam antibiotics degradation has been investigated separately, the degradation of β-lactam antibiotics in the presence of both metal ions and DOM is not well evaluated. DOM tends to form complexes with metal ions [24], which may affect the metal ion-involved catalytic degradation process. Taking Cu(II) as an example, the direct photolysis of Cu(II)-DOM complex generated Cu(I) via ligand-to-metal charge transfer (LMCT) pathway [25]. Also, Cu(I) could be formed via the reduction of Cu(II) by photochemically produced superoxide/hydroperoxyl radicals (O2−/HO2) [26]. Furthermore, the irradiated Cu(II)-DOM complex produced O2− and H2O2,促进 the redox cycle of Cu(II) and Cu(I) [27,28]. As such, the presence of DOM may have a great impact on the Cu(II) catalyzed-hydrolysis and oxidation of β-lactam antibiotics. Without the presence of Cu(II), the Cu(II) activation of DOM is not discerned.

Moreover, nanomolar Cu(II) decreased the 3DOM* oxidative efficiency, because the redox cycling of Cu(II)/Cu(I) reduced partial radical intermediates of phenolic contaminants back to their parent compound [29]. Also, micromolar Cu(II) was found to remarkably inhibit DOM*-sensitized degradation of β-blockers probably due to the quenching of DOM* by Cu(II) [30]. A recent study indicated that the metal–DOM complexation capacity was positively correlated with their capacity of quenching DOM*. The paramagnetic metal ions (e.g. Cu(II), Mn(II) and Fe(III)) exhibited stronger inhibition for 3DOM* than the others (e.g. Ca(II), Al(III) and Zn(II)) [31]. Thus, it is expected that the photodegradation kinetics of β-lactam antibiotics in the co-existence of Cu(II) and DOM, the situation encountering in real waters, would perform differently from that in the presence of NOM only and needs further investigation. Furthermore, the degradation of β-lactam antibiotics in the Cu(II) and DOM co-present waters during the day and night cycles is also of research interests. The dominant contributors for β-lactam antibiotics degradation may be different under light and dark conditions, leading to a different trend in β-lactam antibiotics degradation, which needs to be discerned.

The objectives of this study are to (i) understand the β-lactam antibiotics degradation kinetics in the co-existence of Cu(II) and DOM solutions in the dark and under sunlight irradiation; (ii) reveal the interrelation between Cu(II), DOM and β-lactam antibiotics; (iii) quantify the contributions of 3DOM*, *OH, and 1O2 on β-lactam antibiotics degradation in the absence and presence of Cu(II); (iv) elucidate the multiple influenced pathways of Cu(II) and DOM on β-lactam antibiotics degradation during the day and night cycles. In this study, AMX and AMP are selected as the representative β-lactam antibiotics due to their wide occurrence in the aqueous environment. AMX and AMP have the same β-lactam ring but differ from the phenylglycine primary amine group (Fig. S1 in supporting information). We can also explore the impacts of Cu(II) on the side chain of β-lactam ring through comparing AMX and AMP degradation behaviors. Suwannee River fulvic acid (SRFA) was applied as a typically allochthonous aquatic DOM. Nanomolar concentrations of Cu(II) was applied to represent the environmental concentrations generally occurred in real water.

2. Materials and methods

2.1. Reagents

Ampicillin trihydrate (AMP, >98%) and amoxicillin trihydrate (AMX, >98%) were obtained from TCI Chemicals (Japan). The structure of AMX and AMP were given in Fig. S1. Cu(II) sulfate pentahydrate (Cu(II), >99.999% purity) was obtained from Sigma-Aldrich (USA). SRFA (Cat. NO. 25101F) was purchased from the International Humic Substances Society (IHSS). Furfuryl alcohol (FFA, 98%), terephthalic acid (TA, 98%), 2-hydroxy-terephthalic acid (2HTA, 98%), Amplex Red (AR, >98%), ethylenediaminetetraacetic acid disodium salt (EDTA, >99%) and horseradish peroxidase (HRP) were obtained from Sigma-Aldrich (USA). Acetic acid (Fisher Scientific) and acetonitrile (Merck) were of HPLC grade.

2.2. Experimental procedures

A Q-SUN Xenon Test Chamber (Xe-1) solar simulator equipped with a Daylight-Q optical filter (>290 nm) was employed for sunlight irradiation study. The total absolute irradiance spectrum over the wavelength range 290–800 nm, was measured by using an Ocean Optics USB-4000 spectrometer (Fig. S2). The light intensity between 290 and 400 nm was held at 40 W m−2. The total photon flux was determined to be 4.1 × 10−7 E cm−2 s−1 using p-nitroanisole/pyridine actinometry [32]. In this study, the initial concentration of AMX (or AMP) was 1 μM. The levels of SRFA ranged 0 to 20 mg C L−1, and the concentrations of copper ranged from 0 to 500 nM. The solution pH was adjusted to pH 6.0 using 0.1 M HCl or NaOH. No precipitation of Cu(II) was observed in this study, since most of Cu(II) was present as Cu(II)-DOM complex generated Cu(I) via ligand-to-metal charge transfer (LMCT) pathway [25]. Also, Cu(I) could be formed via the reduction of Cu(II) by photochemically produced superoxide/hydroperoxyl radicals (O2−/HO2) [26]. Furthermore, the irradiated Cu(II)-DOM complex produced O2− and H2O2, promoting the redox cycle of Cu(II) and Cu(I) [27,28]. As such, the presence of DOM may have a great impact on the Cu(II) catalyzed-hydrolysis and oxidation of β-lactam antibiotics. Without the presence of Cu(II), the Cu(II) activation of DOM is not discerned.

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To explore the sorption of AMX (or AMP) on SRFA, centrifugation tests were conducted using a Macrosep Advance Centrifugal Device (1 K, MWCOs, Pall). The cutoff size of the membrane (1 K Da) was sufficiently small to make it impermeable to the larger SRFA components but permeable to the much smaller molecular weight compounds (e.g., AMP, AMX and some micromolecule compounds of SRFA) [20,34]. As illustrated in Fig. S3, the solution containing 10 mg C L−1 SRFA and 100 μM AMX (or AMP) was added into the sample reservoir and centrifuged for 10 min. The addition of Cu(II) up to 500 nM is not expected to affect the adsorption of AMX (or AMP) by SRFA via electrostatic attraction [20,34], thus Cu(II) was not involved in this adsorption experiment. The compounds with molecular weight higher than 1 K Da (SRFA and SRFA-complex) were retained in the sample reservoir, whereas the compounds with the molecular weight less than 1 K Da (AMX or AMP) were collected in the filtrate receiver. Through comparing the initial AMX
or AMP concentration with that in the filtrate after centrifugation, we can calculate the sorption proportion of AMX or AMP on SRFA. The complexation ability of SRFA and AMX/AMP with Cu(II) was also evaluated by adding 50 μM of Cu(II) into the AMX/AMP and SRFA mixture for further centrifugation. The filtrates were scanned the absorbance of filtrate using a UV−vis spectrophotometer (Shimadzu, UV-2700). A higher absorbance means a greater degree of complexation [35].

2.3. Analytical methods

The concentrations of AMX and AMP were analyzed using the HPLC (Dionex Ultimate 3000) with a Synchro C18 column (4.6 × 250 mm, 5 μm) equipped with a diode array detector. For AMX, mobile phase was 20% methanol and 80% H2O (with 0.1% formic acid) and the UV absorbance wavelength was set at 230 nm. For AMP, mobile phase was 50% methanol and 50% H2O (with 0.1% formic acid) and the UV absorbance wavelength was set at 220 nm. The withdrawn sample was detected by HPLC directly without any pretreatment process. The detection limit of both AMX and AMP was 10 nM. H2O2 concentrations were measured according to Amplex Red method [36]. 250 μL samples were collected and mixed well with 250 μL Amplex Red reagent to generate resorufin. The steady-state concentrations of *OH ([*OH]ss) were determined using terephthalic acid (TA) as the probe compound [37]. TA reacted with *OH and produced 2-hydroxy-terephthalic acid (2HTA) [38]. The steady-state concentrations of 1O2 ([1O2]ss) was measured using furfuryl alcohol (FFA, 10 μM) as the probe compound [39]. The concentrations of these probe compounds (resorufin, 2HTA and FFA) were measured using high-performance liquid chromatography (HPLC, Dionex Ultimate 3000). The calculation details were illustrated in Text S1. Error bars in all figures represent the difference in the duplicate tests.

3. Results and discussion

3.1. Effects of Cu(II) on AMX and AMP degradation in dark and light conditions

Fig. 1 displays the observed degradation rates (kobs) of AMX and AMP in the presence of various Cu(II) concentrations in the dark and under sunlight irradiation. The degradation of AMX and AMP obeyed the first-order reaction kinetics. It is interesting to observe the opposite trends of kobs with increasing Cu(II) concentrations in the dark and under sunlight irradiation. As shown in Fig. 1a, kobs of AMP and AMX in the presence of Cu(II) in the dark were enhanced compared to those without Cu(II) present. In the presence of 500 nM Cu(II), kobs of AMP (1.2 × 10−3 h−1) was 6 times of that in the absence of Cu(II) (2 × 10−4 h−1), A similar observation was also observed for AMX. Its kobs (2.3 × 10−3 h−1) was 3 times of that in the absence of Cu(II) (7 × 10−4 h−1) in the dark. These results confirmed Cu(II) was the main contributor for AMX and AMP degradation in the dark condition. Cu(II) complexation is essential for AMX and AMP dark degradation. AMX and AMP undergo catalyzed hydrolysis first and then oxidation of their hydrolysis products by Cu(II) [16]. Fig. 1a shows that AMX degradation rate in the dark was faster than that of AMP, which is consistent with the previous study [16]. Since AMX and AMP share the same primary amine structure in the phenylglycine group, the complexation ability of AMX and AMP with Cu(II) should be similar [40]. AMX and AMP only differ in the AMX containing the phenolic hydroxyl group. Based on the quantitative structure activity relationships (QSARs), the phenolic hydroxyl group is an electron-donating substituent, which may display relatively fast formation of the corresponding phenoxyl radical via Cu(II)-oxidation. Thus, the phenolic hydroxyl group of AMX is conducive to Cu(II) catalyzed oxidation [41].

Fig. 1b shows that both AMP and AMX degraded much faster under sunlight irradiation than in the dark. When Cu(II) was not present in the solution, kobs of AMP under irradiation (2.0 × 10−2 h−1) was two orders of magnitude higher than that in the dark (2 × 10−4 h−1). The degradation rate of AMP (2.2 × 10−2 h−1) was slightly higher than that of AMP under sunlight irradiation. This phenomenon suggests the degradation of AMX was faster than AMP in both light and dark conditions. The irradiated DOM formed a series of oxidative species (1DOM*, *OH, O2 and H2O2), which may contribute to the significant enhancement of AMP and AMX depletions under sunlight irradiation [21,37]. Notably, the presence of Cu(II) significantly inhibited the photo-induced oxidation of AMP and AMX. In comparison to the absence of Cu(II), kobs of AMP and AMX decreased by 39.9% and 38.5% with 500 nM Cu(II) addition, respectively. A similar inhibition impact of Cu(II) was also observed in the photodegradation of 2,4,6-trimethylphenol (TMP) in DOM solutions [29]. Nanomolar Cu(II) primarily restricts 1DOM* electron transfer pathway, thereby decreasing the DOM-mediated photodegradation [29]. In order to illustrate the distinct phenomenon of AMP and AMX degradation in the dark and under irradiation, we evaluate the degradation of AMP and AMX under specific reaction conditions.

3.2. The degradation of AMP and AMX in the dark

Fig. 2 shows that AMP and AMX degraded quickly in the dark when Cu(II) was present in the solution. No observable degradation of AMP and AMX was found in SRFA containing solution in the absence of Cu(II). Notably, the presence of SRFA inhibited the Cu(II)-induced AMX and AMP degradation. As shown in Fig. 2a, a higher SRFA level led to a greater inhibitory effect of AMX degradation. In the presence of 5 mg L−1 and 20 mg L−1 SRFA in the solution, the Cu-catalyzed AMX degradation was inhibited by 6.9% and 12.9%, respectively, after 48 h dark reactions. The inhibition effect of SRFA on AMP degradation in dark was also observed in Fig. 2b. Similarly, the inhibition on AMP degradation was much more pronounced when SRFA concentration was higher. Fig. S4 suggests the sorption of AMP and AMX on SRFA molecule was negligible. However, SRFA competed with AMP or AMX for available Cu(II), because the UV spectral absorbance of samples in the presence of SRFA was generally lower than that in the absence of SRFA (Fig. S5). Therefore, the complexation between SRFA and Cu(II) retarded Cu(II) catalytic degradation of AMX and AMP in the dark condition. Furthermore, comparing Fig. 2a and b, the inhibitory effect of SRFA on AMX degradation was much more evident that on AMP. AMP and AMX share the same β-lactam structure and differ only from the phenylglycine primary amine group. The phenylglycine primary amine group was more susceptible to Cu(II) catalyzed oxidation [15]. The different inhibitory effect of SRFA on AMP and AMX degradation could be attributed to the SRFA depressing Cu(II)-catalyzed oxidation.

Another experiment conducted by adding previously irradiated SRFA into the AMX solutions for dark reactions in the presence of AMP. Interestingly, the pre-irradiated SRFA alleviated the inhibition of SRFA on AMP degradation in the presence of Cu(II). As shown in Fig. S6, AMP degradation rate in the pre-irradiated SRFA solution was 7% higher than that in the un-irradiated SRFA solution after 8 h of dark reactions in the presence of Cu(II). However, the AMX degradation was negligible in pre-irradiated SRFA solutions without Cu(II) presenting (Fig. S6). It could be inferred that the interactions between Cu(II) and irradiated SRFA contributed to the enhancement of AMX degradation. Specifically, Cu(II) oxidized AMX and regenerated Cu(I) (Reaction 1) [17]. In the
**Fig. 1.** The effects of Cu(II) concentration on the AMX and AMP degradation (a) in the dark (b) under sunlight irradiation. Conditions: [AMX] = [AMP] = 1 μM, [SRFA] = 10 mg C L⁻¹, pH = 6.0.

**Fig. 2.** The effects of SRFA on the Cu(II)-catalyzed degradation of (a) AMX and (b) AMP in the dark condition. Conditions: [AMP] = [AMX] = 1 μM, [Cu(II)] = 1 μM, pH = 6.0.
meanwhile, the irradiated SRFA generated H$_2$O$_2$ \((\text{Reactions 2–4})\) [42]. Fig S7 suggests 8 h of pre-irradiated SRFA generated 2.6 $\mu$M of H$_2$O$_2$, which was obviously consumed when AMX and Cu(II) were added into the solution. Although the reactivity between H$_2$O$_2$ and AMX is negligible in the dark [43], the reaction between H$_2$O$_2$ and Cu(I) could proceed to form Cu(III) at pH 6.0 \((\text{Reactions 5})\) [44].

As shown in Fig. S8, the Cu(II)-catalyzed degradation of AMX was accelerated by increasing H$_2$O$_2$ dosages; but it was restrained when catalase was involved.

\[
\text{Cu(II)} + \text{AMP/AMX} \rightarrow \text{Cu(I)} + \text{products} \quad (\text{Reaction 1})
\]

\[
\text{SRFA} \rightarrow \text{SRFA}^- \quad (\text{Reaction 2})
\]

\[
\text{SRFA}^- + O_2 + H^+ \rightarrow \text{SRFA} + HO_2^- \quad (\text{Reaction 3})
\]

\[
\text{HO}_2^- + \text{HO}_2^- \rightarrow \text{SRFA} + H_2O_2 + O_2 \quad (\text{Reaction 4})
\]

\[
\text{Cu(I)} + H_2O_2 \rightarrow \text{Cu(III)} \quad (\text{at pH = 6.0}) \quad (\text{Reaction 5})
\]

**3.3. The photodegradation of AMP and AMX under sunlight irradiation**

When the AMX and AMP solution contained both SRFA and Cu(II), the photodegradation of AMP and AMX may be attributable to the direct photodegradation \((k_d, \text{Cu(II)})\), and indirect photodegradation, which is further classified into the contributions of \(*\text{OH}, *\text{O}_2\), and \(\text{SRFA}^-\) (or expressed as \(3\text{DOM}^-\)), as expressed in Equation (1)–4:

\[
k_{\text{obs}} = k_d + k_{\text{Cu(II)}} + k_{\text{HO}_2} + k_{\text{O}_2} + k_{\text{SRFA}^-} \quad (\text{Equation 1})
\]

\[
k_{\text{Cu(II)}} = k_{\text{obs}}\text{dark} \quad (\text{Equation 2})
\]

\[
k_{\text{OH}} = k_{\text{HO}_2} \frac{[\text{SRFA}^-]}{[\text{OH}]} \quad (\text{Equation 3})
\]

\[
k_{\text{O}_2} = k_{\text{O}_2} \frac{[\text{SRFA}^-]}{[\text{O}_2]} \quad (\text{Equation 4})
\]

Where \(k_d\) used the AMP and AMX degradation rates under direct photolysis in the absence of Cu(II) and DOM. \(k_{\text{Cu(II)}}\) used the observed degradation rates of AMP and AMX in the presence of various Cu(II) concentrations and without DOM present in dark. \(k_{\text{OH}}\) was obtained by multiplying the reaction rates of \(*\text{OH}\) with AMP \((k_{\text{OH}}\text{AMP} = 8.21 \times 10^5 \text{ M}^{-1}\text{s}^{-1})\) or AMX \((k_{\text{OH}}\text{AMX} = 6.49 \times 10^5 \text{ M}^{-1}\text{s}^{-1})\) with experimental measured \([\text{OH}]\). Similarly, \(k_{\text{O}_2}\) was obtained by multiplying the reaction rates of \(O_2\) with AMX and AMP \((k_{\text{O}_2}\text{AMX} = 1.44 \times 10^4 \text{ M}^{-1}\text{s}^{-1})\) with \([\text{O}_2]\). Previous experiments confirmed the reactivity of H$_2$O$_2$ alone with AMX and AMP is negligible, therefore the contribution of H$_2$O$_2$ was not involved (Fig S6, red line). Then \(k_{\text{SRFA}^-}\) was obtained by subtracting the sum of \(k_d, k_{\text{Cu(II)}}\) and \(k_{\text{O}_2}\) from \(k_{\text{obs}}\).

Fig. 3 illustrates the contributions of Cu(II)-catalyzed degradation, direct photolysis and reactive species on the photodegradation of AMX and AMP in the presence of various concentrations of Cu(II). With the increasing Cu(II) concentration from 0 to 500 nM, the contribution of Cu(II)-catalyzed degradation \((k_{\text{Cu(II)}}/k_{\text{obs}})\) increased from 3.2% to 17.3% for AMX (Fig. 3a) and increased from 1.0% to 9.8% for AMP (Fig. 3b), respectively. The observed direct degradation rate of AMX and AMP were \(1.3 \times 10^{-3} \text{ h}^{-1}\) and \(2.0 \times 10^{-3} \text{ h}^{-1}\), respectively, calculated from Fig. S9. Accordingly, the direct photodegradation contribution \((k_d/k_{\text{obs}})\) was more than 98.6% for AMX and was less than 16% for AMP, at Cu(II) levels ranging 0–500 nM.

Indirect photolysis accounts for a large proportion of AMP and AMX degradation (Fig. 3). Fig. S9 illustrated that 21.9% of AMP and 26.8% of AMX were degraded in the presence of 10 mg L$^{-1}$ SRFA after 12-h irradiation, suggesting that SRFA was served as the photosensitizer in AMP and AMX photodegradation. Generally, irradiated SRFA in solution produced \(\text{SRFA}^-\), which could be quenched by dissolved oxygen to form \(\text{O}_2\), with the rate constant of \(8.1 \times 10^{8} \text{ M}^{-1}\text{s}^{-1}\) [46]. \(\text{O}_2\) could also be rapidly consumed by H$_2$O$_2$ with the rate constant of \(7.8 \times 10^3 \text{ s}^{-1}\), as expressed in Reactions 6–8 [47]. In this study, the \([\text{O}_2]_{\text{in}}\) concentrations in the AMX and AMP solutions were around \(10^{-13} \text{ M}\) and showed little variations with increasing Cu(II) concentrations (Table S1). The oxidative ability between \(\text{O}_2\) with AMX and AMP was relatively low (around \(10^{-4} \text{ M}^{-1}\text{s}^{-1}\)). Thus, the contribution by \(\text{O}_2\) was negligible both in the absence and in the presence of Cu(II) (<0.1%).

\[
\text{SRFA} + h\nu \rightarrow \text{SRFA}^- \quad (\text{Reaction 6})
\]

\[
\text{SRFA}^- + \text{O}_2 \rightarrow \text{SRFA} + \text{O}_2 \quad (\text{Reaction 7})
\]

\[
\text{O}_2 + \text{H}_2\text{O} \rightarrow \text{O}_2 + \text{H}_2\text{O}_2 \quad (\text{Reaction 8})
\]

Fig. 3 suggests \(*\text{OH}\) played a minor role in the AMX and AMP photodegradation. Although the irradiated SRFA yielded \(*\text{OH}\), SRFA also quenched \(*\text{OH}\) as well [48]. In the absence of Cu(II), \(*\text{OH}\) generated from irradiated SRFA contributed to 3.0% of AMX and 3.5% of AMP degradation, with the \([\text{OH}]_{\text{in}}\) concentrations of 2.7 \times 10^{-17} for AMX and 2.4 \times 10^{-17} M for AMP, respectively (Fig. 3). In the presence of 500 nM of Cu(II), the \([\text{OH}]_{\text{in}}\) levels in AMX and AMP solution were reduced to \(1.5 \times 10^{-17}\) and \(1.6 \times 10^{-17}\) M, respectively. Overall, the \(*\text{OH}\)-mediated photodegradation pathway had limited influence on AMX and AMP irradiated degradation (<3.9%).

Fig. 3 indicates \(\text{SRFA}^-\) oxidation played a major role. In the absence of Cu(II), the contribution of \(\text{SRFA}^-\) to AMX and AMP was 87.8% and 85.4%, respectively. According to Equation (1)–4, \(k_{\text{SRFA}^-}\) were calculated to be \(1.91 \times 10^2 \text{ h}^{-1}\) for AMX and \(1.71 \times 10^2 \text{ h}^{-1}\) for AMP, respectively. To confirm the roles of \(\text{SRFA}^-\) in AMX and AMP photodegradation, two quenching experiments were conducted using sorbic acid and isopropanol as the \(\text{SRFA}^-\) quenchers [49]. The results shown in Fig. S10 demonstrate that the degradation of AMX and AMP were greatly inhibited when \(\text{SRFA}^-\) quenchers were present. In general, triplet sensitizers react with the organic compounds through both electron transfer or energy transfer pathway [19]. AMX and AMP, owning electron-rich moieties, are particularly susceptible to be oxidized by \(\text{SRFA}^-\) via one-electron transfer process [37]. The oxidation of AMX and AMP by \(\text{SRFA}^-\) firstly yielded radical intermediates (AMX$^+$ or AMP$^+$), and further transformed to the oxidation products, as illustrated in Reactions 9 and 10.

\[
\text{SRFA}^- + \text{AMX/AMP} \rightarrow \text{SRFA}^- + \text{AMX}^+ + \text{AMP}^+ \quad (\text{Reaction 9})
\]

\[
\text{AMX}^+ + \text{AMP}^+ \rightarrow \text{oxidation products} \quad (\text{Reaction 10})
\]

Notably, enhancing Cu(II) concentration significantly reduced the contribution of \(\text{SRFA}^-\) on AMX and AMP photodegradation. When Cu(II) concentrations increased from 0 to 500 nM, the contribution of \(\text{SRFA}^-\) to AMX degradation reduced from 87.8% to 70.2% (Fig. 3a), and decreased from 85.4% to 69.9% for AMP (Fig. 3b).
We suspected that nanomolar Cu(II) depressed the oxidizing capacity of $^{3}$SRFA towards AMX and AMP. To verify this hypothesis, 4-carboxybenzophenone (CBBP) was employed as a model triplet sensitizer to replace SRFA in the solution. Irradiated CBBP could simply generate $^{3}$CBBP, avoiding the interference of $^{1}$O$_{2}$, OH, and other free radicals generated from SRFA [21]. As shown in Fig. S11, increasing Cu(II) concentration significantly reduced the AMX degradation in the CBBP solution, confirming that the triplet electron transfer process was affected. A recent study indicated that nanomolar Cu species had little impacts on the generation and loss of $^{3}$DOM$^*$ [29]. As such, the Cu inhibition of triplet electron transfer pathway may be due to the reducing parts of AMX/AMP radical intermediates back to their parent compounds (AMX and AMP) by the formed Cu(I), as illustrated in Reaction 11, like the case of photooxidation of 2,4,6-trimethylphenol [29].

$$\text{Cu(I)} + \text{AMX}^*/\text{AMP}^* \rightarrow \text{AMX/AMP} + \text{Cu(II)} \quad \text{(Reaction 11)}$$

3.4. The degradation of AMP/AMX under light and dark cycle

In the aqueous environment, natural waters experience light and dark cycles. In order to describe the AMX and AMP degradation in the real environment, we conducted the study for 48 h by keeping the 12-h light and 12-h dark cycle. During the reaction period, the variation in pH was less than ±0.1, and no Cu precipitation was observed. Fig. 4 shows the degradation of AMX and AMP in the two light and dark cycles in the absence and presence of 500 nM Cu(II). In the absence of Cu(II), 29.3% AMP was degraded in the first 12-h light irradiation and <0.9% of AMP degraded during the dark condition. However, the overall degradation of AMP in the first cycle was inhibited by adding Cu(II). In the presence of 500 nM Cu(II), AMP degradation rates were significantly reduced to 13.1% under irradiation, but slightly increased to 3.3% in the first dark incubation in comparison to the condition without Cu(II) present. Relative to the photolysis rate, Cu-catalyzed hydrolysis and oxidation rates of AMX and AMP in the dark were quite low ($10^{-4}$ h$^{-1}$), which only played a minor role (less than 5%) in the first day-night cycle.

The degradation of AMX and AMP in the second light cycle was slower than that in the first light cycle (Fig. 4). When it came to the second 12-h irradiation, the AMP degradation rate was 8.6% and 8.0% in the absence and in the presence of 500 nM of Cu(II), respectively. Meanwhile, less than 0.3% AMP was degraded in the second 12-h dark with and without Cu(II) present. A similar phenomenon was also observed for AMX. After two cycles of light and dark, the overall degradation percentages of AMX were 48% and 32% in the absence and presence of 500 nM Cu(II), respectively, and were 38% and 25% for AMP, respectively. These results indicate that the degradation of AMX and AMP in the natural aqueous environment is mainly attributed to the sunlight irradiation. The main role of Cu(II) was inhibition of the AMX and AMP photosensitized degradation, whereas the Cu(II)-catalyzed hydrolysis and oxidation were not quite pronounced. It should be noted that the tests were conducted under 500 nM Cu(II) and the degradation of AMP and AMX was inhibited by 13–16% during two cycles of day and night compared to that without the presence of Cu(II). In the water with the presence of higher concentrations of Cu(II), the inhibition could become much more evident.

3.5. Proposed mechanism

Fig. 5 summarizes the degradation pathways of AMX/AMP in the SRFA and Cu(II) containing solutions in the dark and sunlight.
Fig. 4. The degradation of AMX and AMP under 48 h of day and night cycle. Conditions: [AMX] = [AMP] = 1 μM, [Cu(II)] = 500 nM, [SRFA] = 10 mg Cl⁻/L, pH = 6.0.

Fig. 5. Scheme displaying the role of Cu(II) and SRFA on the degradation of AMX and AMP under the dark and sunlight conditions.
conditions. Cu(II)-catalyzed degradation of AMX/AMP was dominant in the dark. Cu(II) served as both a hydrolysis catalyst and an oxidant in promoting AMX/AMP degradation. Specifically, the primary amine group of AMX and AMP is crucial for the direct oxidation by Cu(II) and their β-lactam moiety can undergo Cu(II)-catalyzed hydrolysis followed by oxidation of hydrolysis products by Cu(II) [16]. Although the interactions between AMX/AMP and SRFA was negligible, SRFA acted as a competitor of AMX/AMP for Cu(II). The formation of Cu(II)-SRFA complex reduced free Cu(II) levels, thereby inhibiting Cu(II)-catalyzed hydrolysis and oxidation of AMX/AMP. Additionally, the pre-irradiated SRFA produced H2O2, which promoted Cu(II) redox cycle and generated Cu(III), thereby enhancing the degradation of AMP and AMX.

Under sunlight irradiation, SRFA plays an important role in promoting AMX and AMP photocatalytic degradation. Irradiated SRFA generated \( ^{3}\text{SRFA} \). Because AMX and AMP own electron-rich moieties, \( ^{3}\text{SRFA} \) undergo one-electron transfer mechanism rather than energy transfer pathway. It has been reported that the energy transfer mechanism of \( ^{3}\text{SRFA} \) mainly proceed with the compounds owning the conjugated double bond [23]. Additionally, other reactive oxygen species (\(^{*}\text{OH} \) and \(^{1}\text{O}_2 \)) generated from \( ^{3}\text{SRFA} \) slightly contributed to AMX and AMP oxidation. Cu(II) significantly restrained the indirect photochemical degradation of AMX and AMP via inhibiting \( ^{3}\text{SRFA} \) electron transfer pathway. The redox of Cu(II)/Cu(I) would not only quench \(^{*}\text{OH} \), but also reduce the radical intermediates (AMX\(^{+} \) and AMP\(^{+} \)) back to their parent compounds (AMX and AMP). Therefore, Cu(II) significantly inhibited the indirect photochemical degradation of AMX and AMP.

4. Conclusions

This study investigated the degradation of two typical β-lactam antibiotics, AMX and AMP, in the solutions containing SRFA and Cu(II) and in the dark and sunlight irradiation conditions. Photocatalytic degradation of AMX and AMP was much faster than hydrolysis in the dark. In the dark condition, Cu(II) catalyzed hydrolysis and oxidation enhanced the degradation of AMX and AMP. The presence of SRFA depressed Cu(II)-catalyzed degradation of AMX and AMP, due to the formed Cu-SRFA complexes and inhibition of Cu(II) oxidation. In sunlight irradiation, \( ^{3}\text{SRFA} \) served as the predominant oxidative species for AMX and AMP photooxidation. Cu(II) restrained \( ^{3}\text{SRFA} \) electron transfer pathway towards AMX and AMP via Cu(II)/Cu(I) redox reactions. After the two day and night cycles, the inhibition effects of Cu(II) on AMX and AMP degradation was pronounced. The investigation suggested the interactions between metal ions and DOM should be considered in the prediction of environmental fates of β-lactam antibiotics.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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