Science of the Total Environment 420 (2012) 160–167

Contents lists available at SciVerse ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Removal of amoxicillin by UV and UV/H_2O_2 processes

Yeon Jung Jung [a], Wan Gi Kim [a], Yeojoon Yoon [a], Joon-Wun Kang [a,*], Young Min Hong [b], Hyun Wook Kim [b]

[a] Department of Environmental Engineering, YIEST, Yonsei University, 234 Maeji, Heungup, Wonju, 220-710, Republic of Korea
[b] School of Environmental Engineering, University of Seoul, Seoulsiripdaero 163, Dongdaemun-gu, Seoul, 130-743, Republic of Korea

ARTICLE INFO

Article history:
Received 15 July 2011
Received in revised form 27 November 2011
Accepted 3 December 2011
Available online 10 February 2012

Keywords:
Amoxicillin
UV/H_2O_2
Antibacterial activity
Transformation products
Hydroxyl radical

ABSTRACT

The degradation of the β-lactam antibiotic amoxicillin (AM) treated with direct UV-C and UV/H_2O_2 photolytic processes was investigated in the present study. In addition, the antibacterial activity of the solution treated by UV/H_2O_2 advanced oxidation was compared with AM solution treated with ozone. The degradation rate of amoxicillin in both processes fitted *pseudo* first-order kinetics, and the rates increased up to six fold with increasing H_2O_2 addition at 10 mM H_2O_2 compared to direct photolysis. However, low mineralization was achieved in both processes, showing a maximum of 50% TOC removal with UV/H_2O_2 after a reaction time of 80 min (UV dose: 3.8×10^{-3} Einstein L^{-1}) with the addition of 10 mM H_2O_2. The transformation products formed during the degradation of amoxicillin in the UV and UV/H_2O_2 processes were identified by LC-IT-TOF analysis. In addition, microbial growth inhibition bioassays were performed to determine any residual antibacterial activity from potential photoproducts remaining in the treated solutions. An increase of the antibacterial activity in the UV/H_2O_2 treated samples was observed compared to the untreated sample in a time-based comparison. However, the UV/H_2O_2 process effectively eliminated any antibacterial activity from AM and its intermediate photoproducts at 20 min of contact time with a 10 mM H_2O_2 dose after the complete elimination of AM, even though the UV/H_2O_2 advanced oxidation process led to bioactive photoproducts.

1. Introduction

For many years, antibiotics have been a significant group of pharmaceuticals used to prevent human infections. They have also been widely used in veterinary medicine to treat diseases and infections, and as feed additives to promote growth and weight gain in livestock. Antibiotics may be excreted through urine, feces, and manure in both non-metabolized and metabolized forms, which then flow into surface water, groundwater, and drinking water through the water cycle. (Hirsh et al., 1999; Kűmmerer, 2009). As a consequence, a variety of antibiotics have been detected in aquatic environments, such as surface water, groundwater, and influent and finished water in drinking water and sewage treatment plants, in amounts ranging from ng to μg L^{-1} (Hirsh et al., 1999; Heberer, 2002; Kim et al., 2007; Choi et al., 2008; Li et al., 2008). Despite these relatively low levels, the frequent occurrence of antibiotics in the aquatic environment has raised concern about their potential impact on the environment and public health. The bacterial population and aquatic life may be disturbed, and resistance against particular types of antibiotics may be developed by pathogenic bacteria in the environment. Some of the adverse effects caused by antibiotics pollution include aquatic toxicity, the development of resistance in pathogenic bacteria, and genotoxicity (Wollenberger et al., 2000; Halling-Sørensen et al., 2002; Pruden et al., 2006; Kűmmerer and Henninger, 2003; Lindberg et al., 2007).

Amoxicillin (AM) is a drug belonging to the class of β-lactam antibiotics that has a broad spectrum against both Gram-negative and Gram-positive bacteria. AM is one of the top-priority human and veterinary pharmaceuticals, and should receive greater attention in the management of environmental systems in Korea due to its high consumption (KEI, 2006). According to recent reports, approximately 97 tonnes of amoxicillin are produced in Korea (KEI, 2006). Moreover, AM belongs to a group of drugs that are predominantly excreted in an un-metabolized form, and some investigations have reported that AM might present a possible chronic risk in the aquatic environment (Jones et al., 2002; Lee et al., 2008).

In the last years, some researchers have reported effective degradation of AM under different treatment processes. Arslan-Alaton and dogruel (2004) studied a pretreatment of penicillin formulation effluent containing amoxicillin trihydrate and potassium clavulanate by advanced oxidation process. Antreozzi et al. (2005) reported ozonation of amoxicillin in the chemical and kinetic point of view. Elmolla and Chaudhuri (2009, 2010a,b) and Trovó et al. (2011) studied the degradation of AM by UV/ZnO, UV/TiO_2, UV/H_2O_2/TiO_2 photocatalysis, and photo-Feonton process. Homen et al. (2010) also investigated the process optimization of AM degradation by Fenton's oxidation using design experiments. Xu et al. (2011) also

* Corresponding author. Tel.: +82 33 760 2436; fax: +82 33 760 2571.
E-mail addresses: chorong50@gmail.com (Y.J. Jung), darkangel0914@hanmail.net (W.G. Kim), yajoony@yonsei.ac.kr (Y. Yoon), jwk@yonsei.ac.kr (J.-W. Kang), fog2k@korea.com (Y.M. Hong), h_kim@uos.ac.kr (H.W. Kim).

0048-9697/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.scitotenv.2011.12.011

investigated photosensitized degradation of AM in natural organic matter isolate solutions using simulated sunlight.

The UV/H_2O_2 process is one of the UV based advanced oxidation processes (AOPs) and generates the hydroxyl radical (•OH) which has strong oxidizing capabilities. Many studies have illustrated the effectiveness of the UV/H_2O_2 process in the oxidation and mineralization of various organic pollutants and this process has been widely studied for the remediation of both ground and drinking waters (Glaze et al., 1995; Lopez et al., 2003; Vogna et al., 2004; Ikehata et al., 2006; Yuan et al., 2009). Although UV/H_2O_2 advanced oxidation is effective in the mineralization of various organic pollutants, however, it is uncertain whether penicillin antibiotics like amoxicillin can be completely mineralized by this process. It is also unknown whether the process renders amoxicillin and its potential photo-transformation products biologically inactive, as bioactivity is an important parameter to consider when assessing the feasibility of an advanced degradation system.

In general, the biochemical activity of antibiotics on microorganisms comes from their specific moiety and the role of each of these moieties in mediating the specific modes of antibacterial activity exhibited by the corresponding antibacterial structure class. In the case of AM, the antibacterial activity is derived from the β-lactam ring system in its structure, which inhibits cross-linkage between the linear peptidoglycan polymer chains that make up major compounds of the cell walls of both Gram-positive and Gram-negative bacteria, leading to inhibition of the synthesis of bacterial cell walls (Livermore and Williams, 1996).

The aim of this study is to investigate the elimination of AM, and look at changes in its antibacterial activity using direct photolysis and UV/H_2O_2 advanced oxidation processes, and examine the formation of AM intermediates from both processes. Microbial growth inhibition bioassays will also be performed to determine the loss of antibacterial activity in the treated solutions. In addition, as ozone is a strong oxidant and generate •OH by self-decomposition in water (Staehelln and Hoigné, 1982). In this study, the antibacterial potency of AM solutions after ozone treatment is compared with those of UV/H_2O_2 advanced oxidation.

2. Experimental method

2.1. Standards and reagents

Amoxicillin (CAS No. 26787-78-0) and hydrogen peroxide solution (35% w/w) were obtained from Sigma-Aldrich® (St. Louis, USA). Methanol and acetonitrile, used for high performance liquid chromatography (HPLC), were from Fisher Chemicals. Bovine liver catalase (C40, ≤0.2 wt.% thymol) was purchased from Sigma-Aldrich®. Phosphate buffer solution was prepared at pH 7 with potassium dihydrogen phosphate (KH_2PO_4, Oriental Chemical Industries, Korea) and sodium hydroxide (NaOH, Duksan Pure Chemicals, Korea). All other chemicals used in this study were of analytical grade. Stock solution of AM and other solutions were prepared in distilled deionized water (DDI) and diluted as required.

2.2. UV photolysis and UV/H_2O_2 advanced oxidation

The direct UV photolysis and UV/H_2O_2 advanced oxidation experiments were carried out in a cylindrical reactor (Pyrex, 500 mL). A low pressure Hg arc-UV lamp (Pan-ray, UVP Inc.), with a monochromatic emission at 254 nm, was used as the light source, this was covered with a quartz tube (JNC Quartz) and installed in the middle of the reactor. The photon fluence rate and effective light path of the photo-reactor were measured by chemical actinometry, using 0.3 mM and 10 mM H_2O_2 as an actinometer (solution buffered at pH 7 with 1 mM phosphate buffer) (Guittonneau, 1989; Glaze et al., 1995; Kang and Lee, 1997), which were determined to be 8×10^{-7} Einstein L^{-1} s^{-1} (corresponding to 0.38 W L^{-1}) and 5.5 cm, respectively. The temperature of the solution in the reactor was constantly maintained at 20 ± 2 °C using an external water cooling jacket surrounding the reactor. The initial concentration of AM in all test sets was established as 100 μM, in which the pH was adjusted to 7 with 1 mM phosphate buffer solution. For the UV/H_2O_2 runs, H_2O_2 stock solution (0.4, 2, 3, 4, 5 and 10 mM) was added just before exposure to UV radiation. The residual H_2O_2 in the treated sample was immediately quenched by 0.2–2 mg L^{-1} of bovine liver catalase (C40, ≤0.2 wt.% thymol, Sigma-Aldrich®). In addition, in order to confirm that the H_2O_2 in the sample was quenched completely, the residual concentration in the sample was checked at 30 min and 60 min after adding the quenching reagent (i.e. bovine liver catalase) to the sample using an H_2O_2 assay test kit (colorimetric with test strips, Merckoquant®). The AM concentration was analyzed using an HPLC system (Gilson Inc., USA), equipped with a reverse phase column (Waters 5 μm ODS2 4.6×250 mm, C_{18}) and a variable UV/Visible (Vis) wavelength detector. The mobile-phase consisted of 1% acetic acid/acetonitrile (70:30, v/v) with an isocratic flow of 1 mL min^{-1}. AM was detected at 254 nm. The method detection limit (MDL) was 0.24 μM. As the structure of AM contains an amino group and a β-lactam ring, the ammonium ion (NH_4^+) may be released into the solution as AM is degraded. In order to check this, the concentration of ammonium ion was monitored during the run. NH_4^+ in a sample was analyzed using an ion chromatography system (Metrohm 761 compact IC, Switzerland), equipped with an 813 compact auto-sampler (Metrohm, Switzerland) and suppressed conductivity detector, maintained at 25 ± 0.1 °C. The column was a Metrosep C4, 5 μm×4.0 mm×150 mm, packed silica gel/carboxyl group (Metrohm, Switzerland). The data acquisition was performed using the 761 compact IC 1.1 program (Metrohm, Switzerland). The samples were eluted using 1.7 mM HNO_3, delivered at the flow rate of 0.9 mL min^{-1}. The total organic carbon (TOC) was analyzed using a TOC analyzer-TOC − $V_{CPH/CPN}$ (Shimadzu Co, Japan).

2.3. Ozone treatment

AM solutions were prepared at pH 7 using 1 mM phosphate buffer solution with initial concentrations of 100 μM. The solutions were ozonated by injecting various volumes of saturated ozone stock solution (0.9–1.2 mM O_3) via a gas-tight, glass syringe to attain the desired ozone dose in the range from 42 to 146 μM. Then, the solutions were capped tightly and left for one hour to give a sufficient reaction time between AM and ozone. After one hour, the complete decomposition of residual ozone was confirmed by the indigo method. Two 2 mL samples of each solution were collected for analysis of AM concentration by HPLC-UV and the microbial growth inhibition bioassay, respectively.

2.4. Microbial growth inhibition bioassays

The antibacterial activities of the AM solutions, before and after treatment by ozonation, UV photolysis and UV/H_2O_2 advanced oxidation (which served to indicate the solutions' ability for bacterial growth inhibition), were measured using *Escherichia coli* (ATCC 25922) as a test microorganism, according to a protocol based on the procedures described by Wammer et al. (2006) and Suarez et al. (2007). In brief, standard solutions of AM and the samples treated with UV, ozone and UV/H_2O_2 advanced oxidation were prepared in 1 mM phosphate buffer solution at pH 7. Four mL samples were taken from both the standard and the treated sample solutions. From the collected samples, 2 mL aliquots were analyzed using HPLC, to determine the remaining concentration of the parent antibiotic. In addition, positive and negative growth controls were prepared by 100 μM AM solution and 1 mM phosphate buffer solution dosing 1 mL of *E. coli* broth culture in sterile Mueller–Hinton broth, respectively. It was

observed that the bovine liver catalase itself did not affect the growth of *E. coli*. Each of the culture tubes, containing another 2 mL of either standards or treated sample solutions, were subsequently inoculated with 1 mL of a broth *E. coli* culture containing approximately 1×10^6 CFU mL^{-1}. The *E. coli* broth culture was prepared by incubating overnight in sterile Mueller–Hinton broth at 37 °C. Each culture tube was then capped tightly and incubated for 8 h at 37 °C on a shaking incubator rotating at 200 rpm. After the incubation, the bacterial growth was assessed by measuring the optical density at 600 nm and comparing this to the initial optical density of each solution ($8hOD_{600} - initialOD_{600}$). The loss of antibiotic concentration over the 8 hour period was negligible (error < 1%). All bioassays were performed in triplicate. The OD_{600} value was converted to growth inhibition, I, according to the following:

$$I = \frac{A_{max} - A}{A_{max}} \quad (1)$$

where A_{max} represents the maximum absorbance recorded for each sample set (i.e., the absorbance corresponding to uninhibited culture growth, obtained from positive control), where the I value of the samples would vary from 0 to 1 (with a value of 1 indicating complete inhibition and 0 indicating null inhibition).

2.5. Identification of transformation products of AM

The transformation products of AM were separated and identified using a Shimadzu LCMS-IT-TOF (ion trap-time-of flight hybrid mass spectrometer), run by LCMS Solution software (version 3.5SP2). For the chromatographic separation, an HK-21 AIW column (150 mm × 2 mm, 2 μm, Shimadzu Corp.) was used. A gradient elution was performed with a mobile phase of 10 mM ammonium formate (A) and methanol (B) at a flow rate 0.2 mL min^{-1} i.e.: 0–2 min, 5% B; 2–20 min, 30% B; 20–25 min, 30% B; 25–28 min, 95% B; 28–33 min, 95% B; 33–33.01 min, 5% B. The auto-sampler was used to perform5 μL injections, with the samples maintained at 4 °C. The mass spectrometer was operated under the flowing conditions: ion source: electrospray ionization (ESI) in the positive mode, ionization voltage: +4.5 V, ion accumulation time: 30–50 ms, nebulizing gas: N_2, 1.5 L min^{-1}, drying gas: N_2, 15 L min^{-1}, scan range: 100–1000 m/z. Shimadzu's Formula Predictor Software was also used to verify the identification of AM and its by-products.

3. Results and discussion

3.1. Degradation of AM by UV/H_2O_2 advanced oxidation

With UV/H_2O_2 advanced oxidation, AM will be degraded by UV direct photolysis and the OH radical oxidation pathway (Sharpless and Linden, 2003; Pereira et al., 2007). The overall degradation rate of AM is given by:

$$-\frac{d[AM]}{dt} = (k_{OH/AM}[\bullet OH] + k_d)[AM] \quad (2)$$

where $k_{OH/AM}$ is the second-order rate constant of •OH with AM ($k_{OH/AM} = 3.9\times10^9$ M^{-1} s^{-1}, Antreozzi et al., 2005) and k_d is the direct photolysis rate.

The degradation of AM via UV direct photolysis and UV/H_2O_2 advanced oxidation was investigated with the addition of various H_2O_2 concentrations (0, 0.4, 2, 3, 4, 5, and 10 mM H_2O_2), with the results plotted as UV irradiation time (Fig. 1). *Pseudo* first-order kinetics, as given by Eq. (3), were observed for the degradation of

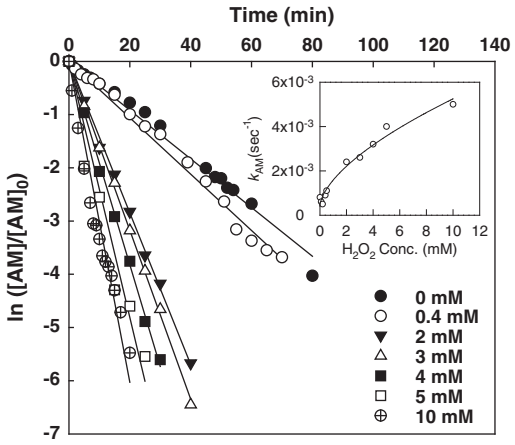

Fig. 1. Degradation of AM as a function of H_2O_2 concentration. The inserted figure indicates the overall-time based rate constants (k'_{AM}) at different H_2O_2 concentration. $[AM]_0 = 100$ μM, pH 7, 20 °C. Legend indicates initial H_2O_2 concentration of the unirradiated samples.

AM, as indicated by the linear relationship between $\ln([AM]/[AM]_0)$ versus UV irradiation time:

$$-\ln\frac{[AM]}{[AM]_o} = k_o t \quad (3)$$

where $k_o = k_{OH/AM}[\bullet OH] + k_d$

Under the same UV dose, the *Pseudo* first-order rate constants, k_o, were greatly increased with increasing H_2O_2 concentrations, in a parabolic pattern, and enhanced up to six-fold with the addition of 10 mM H_2O_2 compared to direct UV photolysis (0 mM H_2O_2).

In this study, the two primary parameters influencing the direct photolysis of AM, i.e. the quantum yield (Φ_{AM}) and molar extinction coefficient (ε_{AM}) at 254 nm, were determined. The value of ε_{AM} at 254 nm was calculated to be 1006 M^{-1} s^{-1}. The quantum yield of AM was determined to be 0.01 mol $Einstein^{-1}$ using a computer-aided program; Poly math software (ver. 6.10, CACHE Co.), using as input the measured parameters of ε_{AM}, A_t, $I_{0,}$ and b, as given by the following equations (Kang and Lee, 1997):

$$-\frac{d[AM]}{dt} = \Phi_{AM} I_0 f_{AM}\left[1 - \exp^{(-2.303A_t)}\right] \quad (4)$$

$$f_{AM} = \frac{\varepsilon_{AM} b[AM]}{\varepsilon_{AM} b[AM] + \sum_{BP} b[BP]} = \frac{\varepsilon_{AM} b[AM]}{A_t} \quad (5)$$

where:

I_o	UV intensity (8×10^{-7} Einstein L^{-1} s^{-1})
Φ_{AM}	Quantum yield (mol $Einstein^{-1}$)
ε_{AM}	Molar extinction coefficient at 254 nm (M^{-1} cm^{-1})
A_t	Total UV absorbance experimentally measured at 254 nm.
b	Light path (5.5 cm)
f_{AM}	Fraction of photons (254 nm) absorbed in the presence of other 254 nm absorbers
BP	By-products formed by UV photolysis

Since oxidation or photolysis is likely to yield the formation of by-products possessing toxicity, the removal of total organic carbon (TOC) may be more important than the removal of the parent compound. Fig. 2 shows the removal of total organic carbon (TOC) as a function of H_2O_2 addition. With direct UV photolysis, only a slight decrease in the TOC (<10%) was observed after 80 min of irradiation; whereas, 50% removal was achieved after 80 min of UV irradiation with the addition of 10 mM H_2O_2. Compared with the degradation rate of AM with the

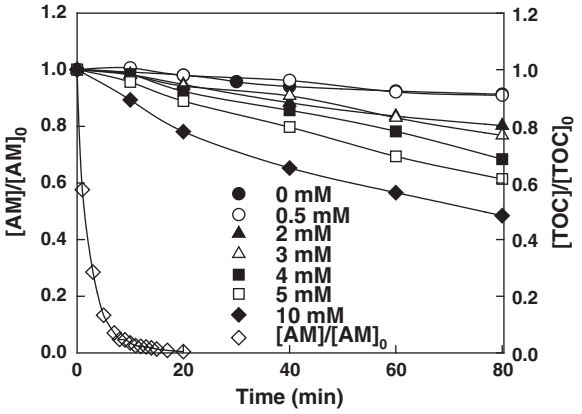

Fig. 2. TOC removals as a function of H_2O_2 addition and degradation of AM at 10 mM H_2O_2 addition; $[AM]_0 = 100$ μM, pH 7, 20 °C. Legend indicates initial H_2O_2 concentration of the unirradiated samples.

addition of 10 mM H_2O_2, even though more than 99% of the AM was degraded in 20 min, only a small (<22%) amount of TOC removal was achieved, implying that the by-products formed from the parent compound remained in the solution.

3.2. Photo-degradation by-products identification

To investigate the formation of by-products during direct photolysis and UV/H_2O_2 advanced oxidation, LCMS-IT-TOF analyses were carried out on reacted samples collected at different irradiation times. The by-products were identified by structural elucidation of their molecular ions and fragments, using ion trap MS and MS/MS experiments. Identity confirmation was provided by accurate mass determination using electrospray ionization (ESI)-IT-TOF MS to deduce their molecular formulae. Fig. 3 shows the mass spectra of the ion trap MS and MS/MS analyses of AM, as well as its chemical structure. The measured mass (m/z) for protonated AM was 366 m/z, with the mass spectrum also showed the potassium adduct and amoxicillin dimer at 404 and 731 m/z, respectively. From the MS/MS analysis of the protonated molecular ion of AM, the fragment ions were measured at 349, 234, 208 and 160 m/z, with the main fragment shown in the mass spectrum at 349 m/z due to the loss of ammonia. From the fragmentation results, the chemical structure of AM was confirmed, and is shown in Fig. 3(b). This mass spectrum and the elucidation of the chemical structure were accurately confirmed using the results obtained by Nägele and Moritz (2005).

Fig. 4 shows the AM degradation and profiles of the by-products formed during the UV alone and UV/H_2O_2 processes. Several unknown by-products ($[M+H]^+ = 160$, 225, 286, 288, 316, 328, 332, 358, and 360 m/z) were also generated, but disappeared on UV irradiation or UV/H_2O_2 advanced oxidation.

Of the unknown peaks detected after both the UV alone and UV/H_2O_2 processes, that with $[M+H]^+ = 160$ m/z had the largest peaks area, with the chemical formula confirmed as $C_6H_{10}NO_2S$ from the MS/MS analysis (daughter ion of 160.0445 m/z: $[M\text{-}COOH]^+ = 114.0372$ m/z). This product at 160 m/z was formed by the cleavage of the β-lactam ring of the parent molecule, and is characteristic of protonated AM (Fig. 5(a)) (Goolsby and Brodbelt, 2008). The mass spectrum and proposed structure of the oxidation intermediate ($[M+H]^+ = 382$ m/z)

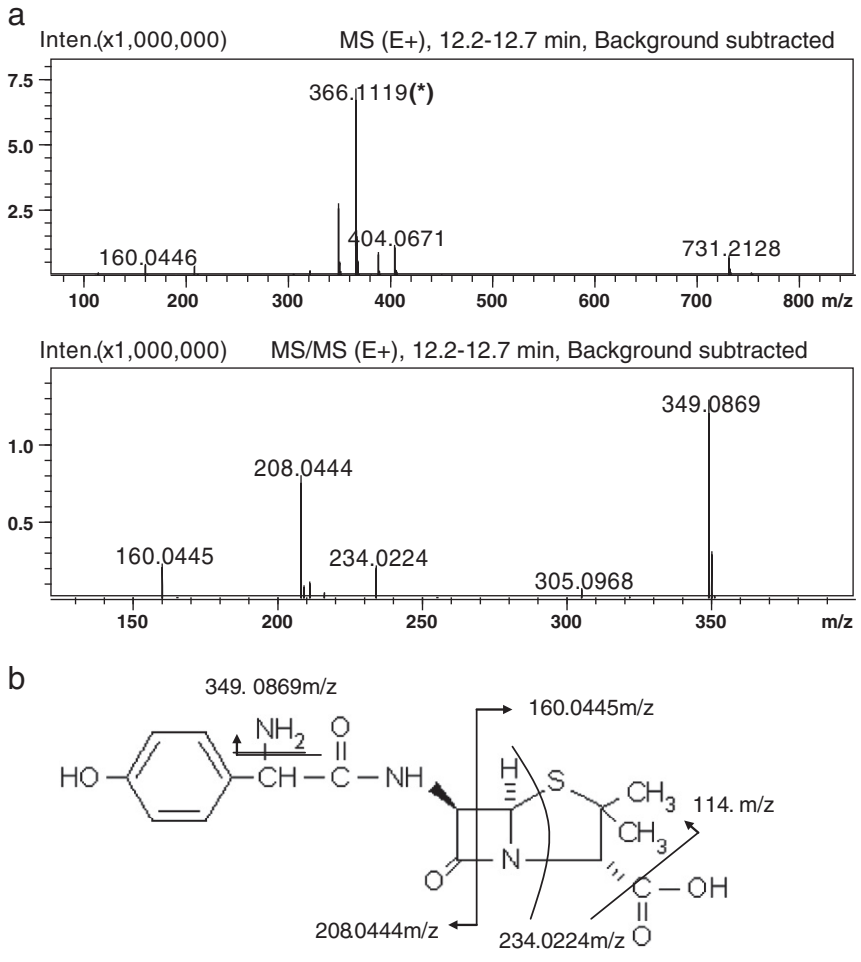

Fig. 3. Mass spectra and chemical structure of amoxicillin; ($C_{16}H_{19}N_3O_5S$), $[M+H]^+ = 366.1119$ m/z, precursor ion = 366.1119 m/z.

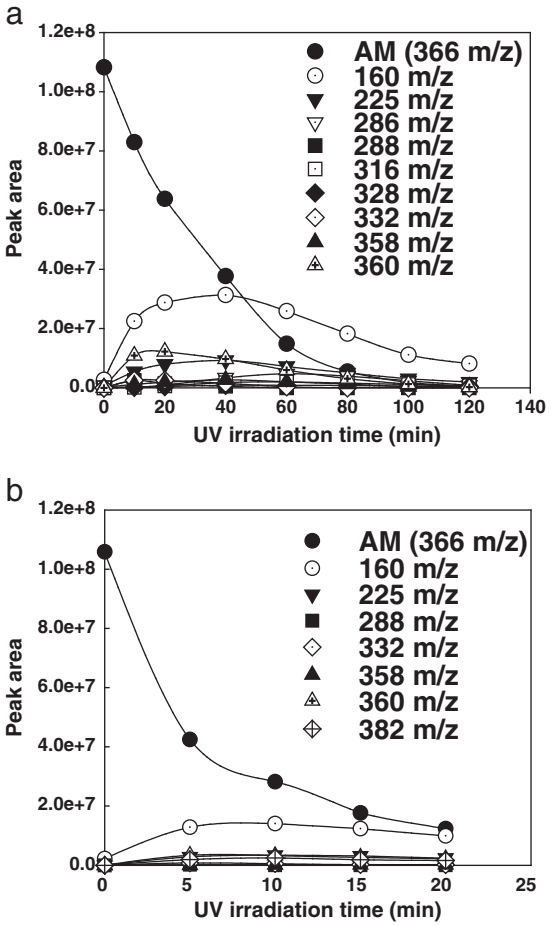

Fig. 4. AM degradation and formation profiles of its by-products by UV irradiation and UV/H_2O_2 oxidation. (a) UV alone (b) UV/H_2O_2 $[AM]_0 = 100$ μM, $[H_2O_2]_0 = 10$ mM.

are shown in Fig. 5(b). The oxidation intermediate ($[M+H]^+ = 382$ m/z) was detected in only the sample treated with UV/H_2O_2, but not in the sample treated with UV alone. The chemical formula of the oxidation product found at 382.1089 m/z was proposed as $C_{16}H_{19}N_3O_6S$, which could be due to a •OH addition reaction of the parent compound, AM. Once •OH fixation occurs at a benzene derivative via a π-complex and a σ-complex, then the •OH group should remain tightly bound (von Sonntag, 2006).

The structural elucidation of this by-product was supported by the fragmentation patterns exhibited by its daughter ions ($[M+H]^+ = 160.0448$ m/z and 365.0822 m/z). Antreozzi et al. (2005) also observed the same peak ($[M+H]^+ = 382$ m/z) during the ozonation of AM.

Fig. 6 shows the changes in the peak area for 382 m/z and the depletion of AM as a function of time for the UV/H_2O_2 advanced oxidation with 10 mM H_2O_2. The peak area increased up to an UV irradiation time of 10 min, but then slowly decreased, such that the product still remained after a reaction time of 20 min, after which more than 90% of AM had been degraded. As previously described, the antibacterial activity of AM is known to be derived from the β-lactam ring (Livermore and Williams, 1996). It should be noted that the hydroxylated AM ($[M+H]^+ = 382.1089$ m/z) formed during the UV/H_2O_2 process could also exhibit antibacterial potential since the β-lactam ring is still present in that compound.

During the experimental run, a significant concentration of ammonium ions (NH_4^+) was also released during the degradation of AM, which contains N and an amine group (Fig. 7). Ammonium ions were detected after both the direct UV and UV/H_2O_2 degradation processes, and on comparing the molar concentrations formed with decreasing AM molar concentration, the NH_4^+ yields with greater than 95% AM parent compound degradation were 35, 30, 37, 36, 32, 34 and 42% for additions of 0 (i.e. UV alone), 0.5, 2, 3, 4, 5 and 10 mM H_2O_2, respectively. The concentration of NH_4^+ continuously increased, even after the complete elimination of AM. This suggested further degradation of N-containing intermediates occurred for the duration of the photodegradation process.

3.3. Changes of antibacterial activity after UV and UV/H_2O_2 advanced oxidation

The residual antibacterial activities, I, of the UV, UV/H_2O_2 advanced oxidation and ozone-treated AM solutions were examined by comparing their ability to inhibit the growth of *E. coli* with the inhibitory activities measured for various concentrations (24 nM–200 μM) of standard solutions, based on the residual concentration of AM. The dose–response relationships observed for the UV and UV/H_2O_2-treated samples and the standard series (in terms of *E. coli* growth inhibition (I) versus each AM concentration) are depicted in Fig. 8 (a). If the by-products contained in the treated samples possessed any antibacterial activity then the curves would be shifted to the left. As demonstrated in Fig. 8(a), when the antibacterial activity of the AM standard was compared with those obtained from direct UV photolysis and UV/H_2O_2 advanced oxidation (0.4, 4 and 10 mM H_2O_2) it was found that the growth inhibition curves of the treated set shifted to the left compared to those of the standard set. Moreover, on increasing the initial H_2O_2 concentration from 0.4 to 10 mM, the measured antibacterial activity curves of the treated solutions shifted even more to the left. However, a different result was observed with the ozone alone treatment. Unlike the UV irradiation and UV/H_2O_2 advanced oxidation, the two curves for the ozonated AM sample and the standard samples (i.e. untreated samples) almost overlapped, implying that the ozonated AM by-products do not possess any notable residual toxicity (Fig. 8(b)).

The degree of the shift could be quantitatively measured by calculating the EC_{50} values, the median (50%) effective concentration of *E. coli* growth inhibition (Fig. 8(c)). The EC_{50} values of the samples treated by each of the processes clearly showed disagreement between the curves obtained for the standard and samples treated with UV photolysis and UV/H_2O_2 advanced oxidation. In relation to the EC_{50} value of the ozonated sample, there were no appreciable differences in the EC_{50} values between the AM standard (7.01 (±0.06) μM) and the ozonated sample (6.49 (±0.88) μM). These close agreements between the standard and ozonated samples imply that the antibacterial activity of the treated solution originated from the parent compound itself, and that ozonation could be an effective process for the elimination of both the molecule of AM and the antibacterial activity. However, the lower EC_{50} values of 3.60 (±0.08), 3.75 (±0.20), 2.29 (±0.08) and 1.64 (±0.03) μM with additions of 0 (UV direct photolysis), 0.4, 4, and 10 mM H_2O_2, respectively, were observed. Moreover, the EC_{50} values decreased with increasing H_2O_2 concentration. The H_2O_2 solution itself was confirmed not to influence the growth of *E. coli*. As described above, AM would be degraded by •OH oxidation and UV direct photolysis, and the degree of the contributions of the two pathways would vary depending on the H_2O_2 concentration. These results of bacterial growth inhibition implied that the by-products of AM formed by UV photolysis and •OH involved UV/H_2O_2 advanced oxidation also carry antibacterial activity just like their parent compound, AM.

Dodd et al. (2009) observed similar results with ozonation and γ-ray irradiation of the antibiotics, penicillin G (PG) and cephalexin (CP), which also contain a β-lactam ring in their structures, using *Bacillus subtilis* as a probe microbe. They reported that biologically active products of PG and CP may be formed in the reaction of β-lactam antibiotics with •OH. The antibacterial activity of AM is

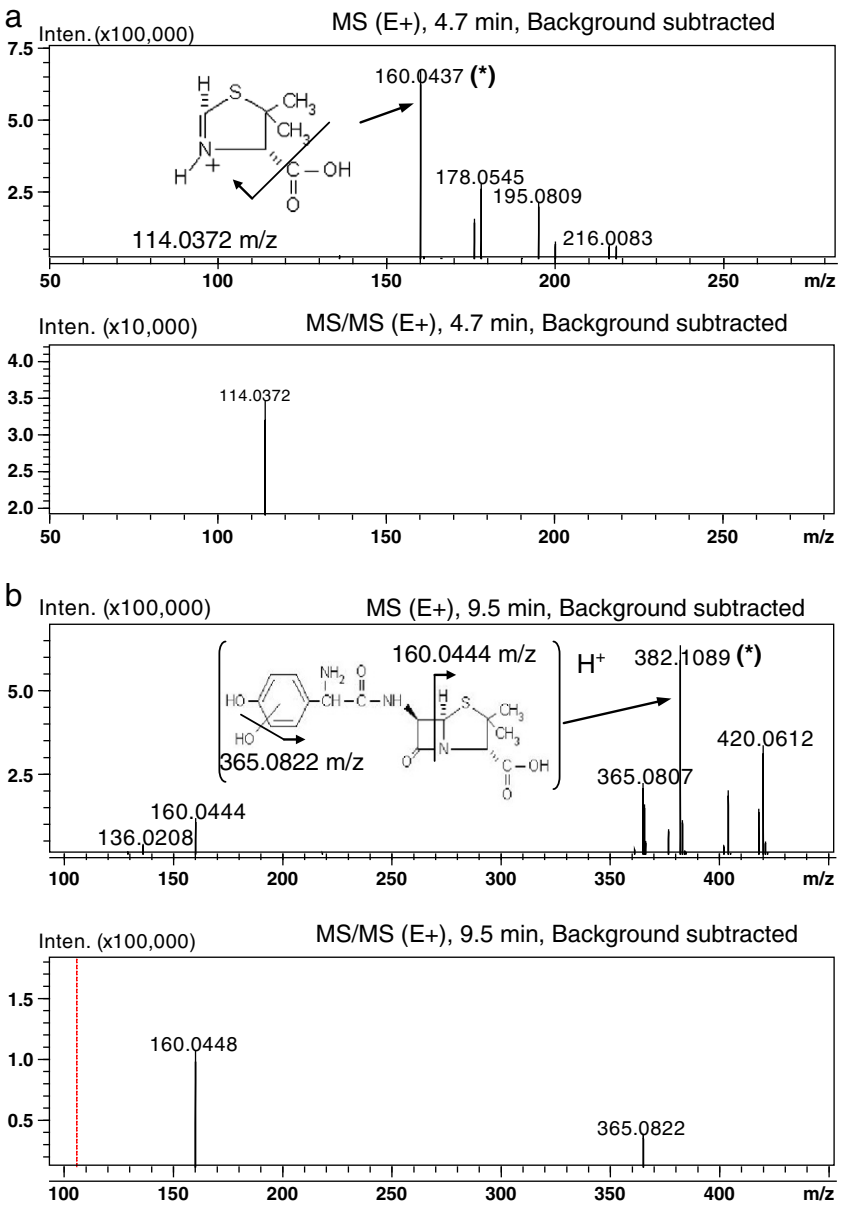

Fig. 5. MS Spectra and the proposed structures of by-products identified in this study. (a) $[M+H]^+ = 160$ m/z (b) $[M+H]^+ = 382$ m/z.

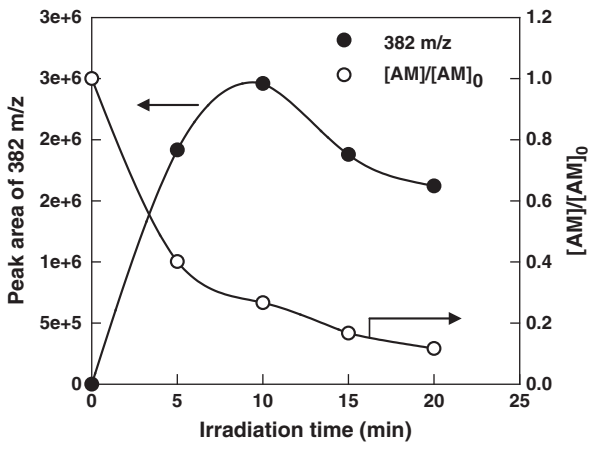

Fig. 6. Change of peak area of 382 m/z and the depletion of AM as a function of irradiation time in UV/H_2O_2 advanced oxidation. $[AM]_0 = 100$ μM and $[H_2O_2]_0 = 10$ mM.

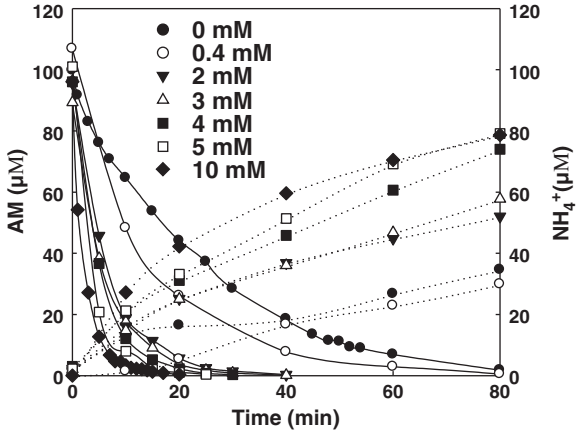

Fig. 7. Degradation of AM and the formation of NH_4^+ as a function of H_2O_2 concentration. Legend indicates initial H_2O_2 concentration of the unirradiated samples. $[AM]_0 = 100$ μM.

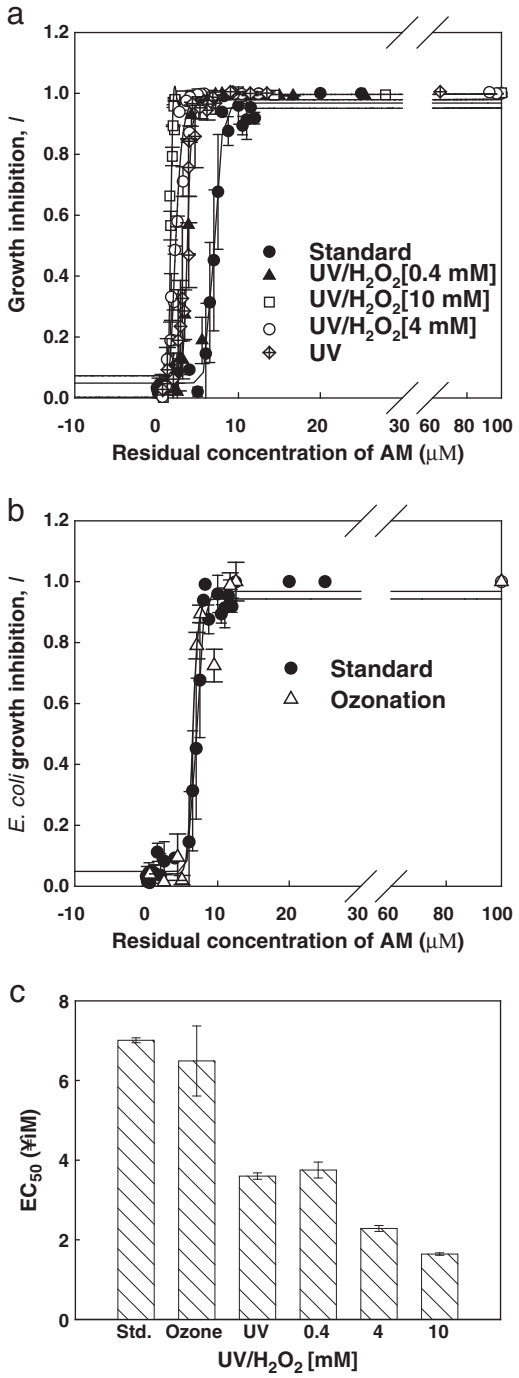

Fig. 8. Change in growth inhibition for *E. coli* in AM samples treated with direct UV photolysis, UV/H_2O_2 advanced oxidation and ozone versus a standard solution derived from a control containing untreated AM solution. (a) UV and UV/H_2O_2, (b) ozone and (c) EC_{50} values. The error bars in the figures indicate standard deviations of replicate experiments.

known to be derived from the β-lactam ring system, which is directly involved in biochemical targets in bacterial cells (Livermore and Williams, 1996; Dodd et al., 2006). Therefore, the presence of by-products which possess the β-lactam ring structure can potentially exhibit antibacterial properties and the identification and bioactivity of such photoproducts needs to be addressed.

In fact, hydroxylated AM (m/z = 382) was identified in the irradiated mixture from the UV/H_2O_2 advanced oxidation process and remained in the treated samples even after 90% of the parent amoxicillin was removed. As the β-lactam ring structure is still present in hydroxylated AM, it is likely that this particular compound is responsible for the observed increased antibacterial activity from the UV/H_2O_2 treated samples, even though the antibacterial activity of hydroxylated AM itself was not measured in the present study. In addition to hydroxylated AM, several other unknown by-products also possess the β-lactam ring structure; these photoproducts could potentially contribute to the overall antibacterial potency of the irradiated mixture. Further research is necessary to better characterize the bioactivity of these unknown photoproducts.

For practical application, full mineralization of antibacterial compounds is likely to be unfeasible from an economic perspective, and may not be an absolute requirement in both drinking water and wastewater treatment. In water treatment, complete elimination of an antibiotic compound, as well as its antibacterial potency, under optimum operational conditions is perhaps a more important goal. The growth inhibition curves shown in Fig. 8(a) were re-plotted as a function of the reaction time in Fig. 9. From the time-based comparison, the antibacterial activity of AM ($[M]_0 = 100\ \mu M$) in UV/H_2O_2 advanced oxidation with 10 mM H_2O_2 was completely eliminated within a quarter of the time required for the UV direct photolysis (0 mM H_2O_2), even though •OH induced bioactive by-products via UV/H_2O_2 advanced oxidation. Moreover, the growth inhibition of the AM solution appeared to decrease toward zero with higher •OH exposure.

In conclusion, biologically active by-products of AM could be formed via direct photolysis or •OH oxidation in the UV/H_2O_2 advanced oxidation process; it will be necessary to pay keen attention and conduct to further investigation of both the by-products formed and their antibacterial potencies. However, the antibacterial potencies resulting from the by-products could be completely eliminated with sufficient reaction time, with the complete degradation of the parent compound, AM. It is therefore vital that appropriate treatment times and adequate amounts of H_2O_2 are employed in the UV/H_2O_2 advanced oxidation process to achieve complete removal of antibacterial activity from an irradiated antibiotic mixture.

4. Conclusions

The degradation of AM and subsequent elimination of antibacterial activity was investigated via direct UV photolysis and UV/H_2O_2 advanced oxidation processes. *Pseudo* first-order kinetics were observed for the degradation of AM via both the direct photolysis and UV/H_2O_2 advanced oxidation processes. With the same UV dose, the rate constants ($k'_{d,AM}$) were greatly increased with increasing H_2O_2 concentration, and enhanced up to six-fold with the addition of 10 mM H_2O_2 compared to

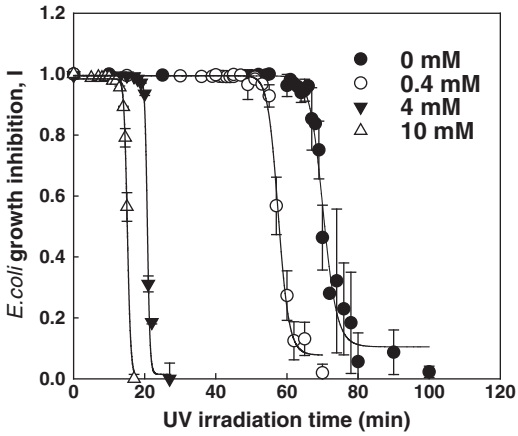

Fig. 9. Time based comparison of *E. coli* growth inhibition curves for the treated samples with UV/H_2O_2 as a function of H_2O_2 addition; $[AM]_0 = 100\ \mu M$. Legend indicates initial H_2O_2 concentration of the unirradiated samples.

direct photolysis (0 mM H_2O_2) alone. However, the mineralization of the AM solution was significantly low, despite the high degradation (>99%) of the parent compound, AM. In a microbial growth inhibition test, ozone treatment completely eliminated both the AM molecules and their antibacterial activity; on the other hand, by-products generated via both direct UV photolysis and UV/H_2O_2 advanced oxidation exhibited antibacterial activity in a similar fashion to parent AM. The hydroxylated and several unknown by-products thought to posses the β-lactam structure were postulated to contribute to the antibacterial activities observed in the UV/H_2O_2-treated samples.These biologically active by-products of AM were found to be formed via direct photolysis or •OH oxidation, In the UV/H_2O_2 advanced oxidation process, complete removal of antibacterial activity was achieved when amoxicillin was irradiated in the presence of 10 mM of H_2O_2 for 20 min. However, complete mineralization was not observed with any of the advanced degradation experiments carried out in the present study. Further investigations to determine the identity and bioactivity of the observed unknown by-products produced in the irradiation processes would be necessary before direct UV photolysis and UV/H_2O_2 advanced oxidation processes can be deemed suitable and feasible for the treatment of wastewater. In addition, it will be necessary to investigate the effect of dissolved organic matter as a representative of natural water in the removal and mineralization of AM by the processes used in this study.

Acknowledgments

This study was supported by the Mid-career Researcher Program (2010-0014829) and Brain Korea 21 (BK21) Program through the National Research Foundation (NRF) grant funded by the Ministry of Education, Science, and Technology (MEST), Republic of Korea.

References

Antreozzi R, Canterino M, Marotta R, Paxeus NA. Antibiotic removal from wastewaters: the ozonation from wastewater. J Hazard Mater 2005;122:243–50.
Arslan-Alaton I, Dogruel S. Pre-treatment of penicillin formulation effluent by advanced oxidation processes. J Hazard Mater B 2004;112:105–13.
Choi KH, Kim YH, Park JI, Park CK, Kim MY, Kim HS, et al. Seasonal variations of several pharmaceutical residues in surface water and sewage treatment plants of Han River, Korea. Sci Total Environ 2008;405:120–8.
Dodd MC, Buffle MO, von Gunten U. Oxidation of antibacterial molecules by aqueous ozone: moiety-specific reaction kinetics and application to ozone-based wastewater treatment. Environ Sci Technol 2006;40:1969–77.
Dodd MC, Kohler HE, von Gunten U. Oxidation of antibacterial compounds by ozone and hydroxyl radical: elimination of biological activity during aqueous ozonation processes. Environ Sci Technol 2009;43. 2498-04.
Elmolla ES, Chaudhuri M. Degradation of the antibiotics amoxicillin, ampicillin and cloxacillin in aqueous solution by the photo-Fenton process. J Hazard Mater 2009;172:1476–81.
Elmolla ES, Chaudhuri M. Degradation of amoxicillin, ampicillin and cloxacillin antibiotics in aqueous solutions by the UV/ZnO photocatalytic process. J Hazard Mater 2010a;173:445–9.
Elmolla ES, Chaudhuri M. Photocatalytic degradation of amoxicillin, ampicillin and cloxacillin antibiotics in aqueous solution using UV/TiO_2 and UV/H_2O_2/TiO_2 photocatalysis. Desalination 2010b;252:46–52.
Glaze WH, Lay Y, Kang JW. Advanced oxidation processes. A kinetic model for the oxidation of 1, 2-Dibromo-3-chloropropane in water by the combination of hydrogen peroxide and UV radiation. Ind Eng Chem Res 1995;34:2314–23.
Goolsby BJ, Brodbelt JS. Characterization of β-lactams by photodissociation and collision-activated dissolution in a quadruple ion trap. J Mass Spec 2008;33:705–12.
Guittonneau S. Contribution á 1'Etude de la Photooxidation de Quelques Micropollutants Organochlor6s en Solution Aqueuseen Presence de Peroxide d'Hydrogen - Comparaison des Systemes Oxydants: H_2O_2/UV, et O_3/H_2O_2. Ph.D. Thesis, University of Poitiers, Poitiers, 1989. France.
Halling-Sørensen B, Sengeløv G, Tjørnelund J. Toxicity of tetracyclines and tetracycline degradation products to environmentally relevant bacteria including selected tetracycline-resistant bacteria. Ach Environ ContamToxicol 2002;42:263–71.
Heberer T. Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: a review of recent research data. Toxicol Letters 2002;131:5-17.
Hirsh R, Terns T, Haberer K, Kratz KL. Occurrence of antibiotics in the aquatic environment. Sci Total Environ 1999;225:109–18.
Homen V, Alves A, Santos L. Amoxicillin degradation at ppb levels by Fenton's oxidation using design of experiments. Sci Total Environ 2010;408:6272–80.
Ikehata K, Naghashkar NJ, El-Din MG. Degradation of aqueous pharmaceuticals by ozonation and advanced oxidation processes: a review. Ozone Sci Eng 2006;28. 353-14.
Jones OA, Vouvoulis N, Lester JN. Aquatic environmental assessment of the top 25 English prescription pharmaceuticals. Water Res 2002;36:5013–22.
Kang JW, Lee KH. A kinetic model of the hydrogen peroxide/UV process for the treatment of hazardous waste chemicals. Environ Eng Sci 1997;14:183–92.
Kim SD, Cho JW, Kim IS, Vanderford BJ, Snyder SA. Occurrence and removal of pharmaceuticals and endocrine disruptors in South Korea surface, drinking, and waste waters. Water Res 2007;41:1013–21.
Korea Environment Institute (KEI). An approach for developing aquatic environmental risk assessment framework for pharmaceuticals in Korea; 2006. RE-05.
Kűmmerer K. Antibiotics in the aquatic environment: a review—part I. Chemosphere 2009;75:417–34.
Kűmmerer K, Henninger A. Promoting resistance by the emission of antibiotics from hospitals and households into effluents. Clinic Microbio Infec 2003;9:1203–14.
Lee YJ, Lee SE, Lee DS, Kim YH. Risk assessment of human antibiotics in Korean aquatic environment. Environ Toxicol Pharm 2008;26:216–21.
Li D, Yang M, Hu J, Zhang Y, Chang H, Jin F. Determination of penicillin G and its degradation products in a penicillin production wastewater treatment plant and the receiving river. Water Res 2008;42:307–17.
Lindberg RH, Björklund K, Rendahl P, Johansson MI, Tysklind M, Andersson BAV. Environmental risk assessment of antibiotics in the Swedish environment with emphasis on sewage treatment plants. Water Res 2007;41:613–9.
Livermore DM, Williams JD. In: Lorian W, editor. Antibiotics in laboratory medicine. 4 ed. Baltimore, MD: Williams and Wilkins; 1996. p. 502–78.
Lopez A, Bozzi A, Mascolo G, Kiwi J. Kinetic investigation on UV and UV/H_2O_2 degradations of pharmaceutical intermediates in aqueous solution. J Photochem Photobio A 2003;156:121–6.
Nägele E, Moritz R. Structural elucidation of degradation products of the antibiotic amoxicillin with ion trap MSn and accurate mass determination by ESI TOF. J Am Soc Mass Spec 2005;16:1670–6.
Pereira VJ, Linden KG, Weinberg HS. Evaluation of UV irradiation for photolytic and oxidative degradation of pharmaceutical compounds in water. Water Res 2007;41:4413–23.
Pruden A, Pei R, Storteboom H, Carson KH. Antibiotic resistance genes as emerging contaminants: studies in Northern Colorado. Environ Sci Technol 2006;40:7445–50.
Sharpless CM, Linden KL. Experimental and model comparisons of low- and medium-pressure Hg lamps for the direct and H_2O_2 assisted UV photodegradation of *N*-nitrosodimethylamine in simulated drinking water. Environ Sci Technol 2003;37: 1933–40.
Staehelln J, Hoigné J. Decomposition of ozone in water: rate of initiation by hydroxide ions and hydrogen peroxide. Environ Sci Technol 1982;16:676–81.
Suarez S, Dodd MC, Omil F, von Gunten U. Kinetics of triclosan oxidation by aqueous ozone and consequent loss of antibacterial activity: relevance to municipal wastewater ozonation. Water Res 2007;12:2481–90.
Trovó AG, Nogueira RFP, Agűera A, Fernandez-Alba AR, Malato S. Degradation of the antibiotic amoxicillin by photo-Fenton process—chemical and toxicological assessment. Water Res 2011;45. 1394-02.
Vogna D, Marotta R, Napolitano A, Andreozzi R, d'Ischia M. Advanced oxidation of the pharmaceutical drug diclofenac with UV/H_2O_2 and ozone. Water Res 2004;38:414–22.
von Sonntag C. Free-radical-induced DNA damage and its repair: a chemical perspective. 1st ed. Germany: Springer-Verlag Berlin Heidelberg; 2006.
Wammer KH, Lapara TM, McNeill K, Arnold WA, Swackhamer DL. Changes in antibacterial activity of triclosan and sulfa drugs due to photochemical transformations. Environ Toxicol Chem 2006;25:1480–6.
Wollenberger L, Halling-Sørensen B, Kusk KO. Acute and chronic toxicity of veterinary antibiotics to *Daphnia magna*. Chemosphere 2000;40:723–30.
Xu H, Cooper WJ, Jung J, Song W. Photosensitized degradation of amoxicillin in natural organic matter isolate solutions. Water Res 2011;45:632–8.
Yuan F, Hu C, Hu X, Qu J, Yang M. Degradation of selected pharmaceuticals in aqueous solution with UV and UV/H_2O_2. Water Res 2009;43:1766–74.