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ARTICLE *in* ENVIRONMENTAL SCIENCE & TECHNOLOGY · JULY 2014

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Ferrate(VI) Oxidation of β-Lactam Antibiotics: Reaction Kinetics, Antibacterial Activity Changes, and Transformation Products

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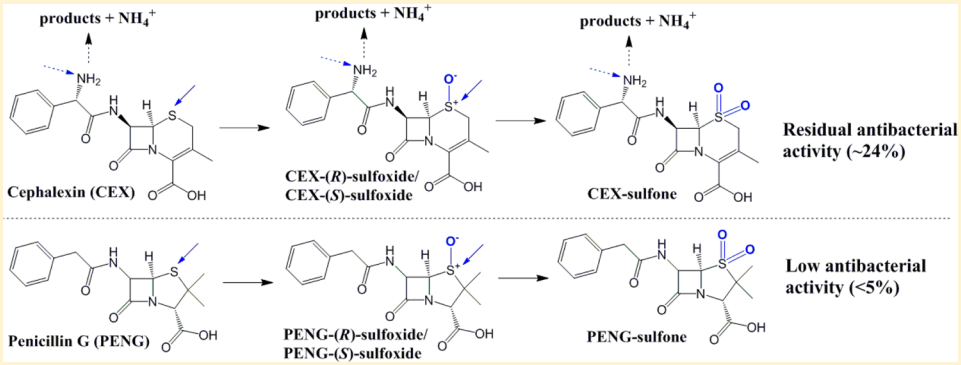
Supporting Information



ABSTRACT: Oxidation of β-lactam antibiotics by aqueous ferrate(VI) was investigated to determine reaction kinetics, reaction sites, antibacterial activity changes, and transformation products. Apparent second-order rate constants (k_{app}) were determined in the pH range 6.0−9.5 for the reaction of ferrate(VI) with penicillins (amoxicillin, ampicillin, cloxacillin, and penicillin G), a cephalosporin (cephalexin), and several model compounds. Ferrate(VI) shows an appreciable reactivity toward the selected β-lactams (k_{app} for pH 7 = 110−770 M^{-1} s^{-1}). The pH-dependent k_{app} could be well explained by considering species-specific reactions between ferrate(VI) and the β-lactams (with reactions occurring at thioether, amine, and/or phenol groups). On the basis of the kinetic results, the thioether is the main reaction site for cloxacillin and penicillin G. In addition to the thioether, the amine is a reaction site for ampicillin and cephalexin, and amine and phenol are reaction sites for amoxicillin. HPLC/MS analysis showed that the thioether of β-lactams was transformed to stereoisomeric (*R*)- and (*S*)-sulfoxides and then to a sulfone. Quantitative microbiological assay of ferrate(VI)-treated β-lactam solutions indicated that transformation products resulting from the oxidation of cephalexin exhibited diminished, but non-negligible residual activity (i.e., ∼24% as potent as the parent compound). For the other β-lactams, the transformation products showed much lower (<5%) antibacterial potencies compared to the parent compounds. Overall, ferrate(VI) oxidation appears to be effective as a means of lowering the antibacterial activities of β-lactams, although alternative approaches may be necessary to achieve complete elimination of cephalosporin activities.

INTRODUCTION

Effluents of municipal wastewater treatment plants (WWTPs) have been shown to contain trace levels of organic micropollutants (ng/L to μg/L range), some of which can be problematic in aquatic ecosystems or to humans.[1,2] Among micropollutants, the presence of various classes of antibiotics in wastewater effluents is an increasing concern, as they can exert selective pressure on microbial communities and cause proliferation of antibacterial resistance among human pathogens.[3,4] To minimize the input of such problematic micropollutants to water resources, an upgrade of WWTPs beyond conventional biological treatments has been proposed and tested in several countries.[5−13]

Ozonation is a promising enhanced wastewater treatment technology for micropollutant elimination.[5,6,8−10,14] Previous studies have shown that significant elimination of many micropollutants and reduction of in vitro and in vivo toxicities can be achieved at reasonable ozone doses in wastewater effluents.[15−17] Nevertheless, widespread application of wastewater ozonation may potentially be limited under certain conditions by formation of toxic byproducts such as bromate or

Received: June 11, 2014
Revised: July 28, 2014
Accepted: July 29, 2014
Published: July 29, 2014

nitrosamines,[14,18] or increases in nonspecific mutagenicity and/or genotoxicity,[19] which requires further investigations.

As an alternative, ferrate(VI) (Fe(VI)) has been shown to be a potential tool to eliminate micropollutants by oxidation and remove phosphate by ferric-phosphate precipitation in a single treatment step.[20] Fe(VI) also shows low reactivity to bromide,[21] and therefore has the advantage of not producing toxic bromate or bromo-organic byproducts. Understanding the aqueous chemistry of Fe(VI) reactions is a prerequisite for a wide application of Fe(VI) in water treatments. Kinetics, products, and mechanisms have been studied for the reaction of Fe(VI) with a variety of simple organic (e.g., phenol[22,23] and aniline[24]) and inorganic compounds (e.g., cyanide,[25] hydroxylamine,[26] and sulfite[27]). Fe(VI) reactions with a number of organic micropollutants have also been investigated.[20,28−36] These studies have shown that organic micropollutants containing phenol-, amine-, olefin-, or thioether-moieties can be potentially transformed during water treatment with Fe(VI). Despite recent advances in the understanding of aqueous Fe(VI) chemistry, detailed knowledge is still lacking for Fe(VI) reactions with structurally complex organic micropollutants with respect to reaction kinetics, pathways, and transformation products. Biological activity (e.g., toxicity) of transformation products from Fe(VI) oxidation is also poorly understood.

The β-lactams are the most frequently utilized family of antibacterial compounds worldwide.[37,38] Among several major classes of β-lactams, penicillins and cephalosporins are the most prominent.[37,38] All penicillins share the penam structure, a five-membered 3,3-dimethyl-4-thiaheptane-2-carboxylic acid system fused to a four-membered β-lactam ring, which is essential for their antibacterial activity.[39] Depending on the side chain (R) that is connected to the β-lactam ring by an amide bond, various penicillins exist. In contrast to the penicillins, cephalosporins contain a six-membered 3-methyl-5-thiahex-2-ene-2-carboxylic acid system. Table S1 of the Supporting Information (SI) shows the structures of the penicillin and cephalosporin compounds selected in this study.

Despite the high usage of penicillins and cephalosporins, relatively low concentrations have been reported for these compounds in some wastewater effluents (<0.05 μg/L),[40−42] which has typically been attributed to facile hydrolysis of the β-lactam ring. However, substantial concentrations of amoxicillin (up to 0.19 μg/L)[43,44] or cephalexin (up to 1.8 μg/L)[45] were detected in other wastewater effluents, which could be due to their more hydrolysis-resistant structures. Considering that many structural analogues of penicillins and cephalosporins are designed to be hydrolysis-resistant,[37−39] β-lactams may still contribute significantly to the overall antibacterial activity observed in wastewater effluents.

Reactions of certain β-lactams with oxidants such as bromine,[46] chlorine,[47] chlorine dioxide,[48] Fe(VI),[49] ozone,[50−52] hydroxyl radicals,[50−53] and sulfate radicals[54] have been investigated to assess the potential for eliminating these compounds during water treatment. Generally, such investigations have focused primarily on assessment of reaction kinetics (e.g., measurement of rate constants and/or removal efficiencies in real water matrixes), and only a few studies addressed transformation products and changes in biological activities.[51,52] In the case of Fe(VI), second-order rate constants have been measured for amoxicillin and ampicillin as a function of pH.[49] However, relatively little is known concerning the identities and properties of transformation products resulting from reactions of Fe(VI) with such β-lactams, and no information appears to be available pertaining to β-lactams exhibiting a broader variety of structural variations.

In this study, amoxicillin (AMX), ampicillin (AMP), cloxacillin (CLOX), and penicillin G (PENG) were selected as penicillins, and cephalexin (CEX) as a cephalosporin. For these compounds, second-order reaction rate constants with Fe(VI) were determined as a function of pH, and subsequently used to determine specific rate constants for reactions between Fe(VI) and β-lactam acid−base species. Fe(VI) reaction kinetics for substructure compounds representing the theorized reactive moieties of the β-lactams were also investigated to facilitate assignment of calculated species-specific reactivity to individual reactive moieties. Oxidative elimination of the β-lactams was also investigated in a wastewater effluent matrix at varying pH and Fe(VI) dose. Major transformation products generated upon treatment of PENG and CEX with Fe(VI) were investigated using HPLC/MS. Reaction pathways and mechanisms were postulated based on the obtained data. Finally, the antibacterial potencies of transformation products resulting from reactions of Fe(VI) with PENG, CLOX, AMX, and CEX were determined.

EXPERIMENTAL SECTION

Standards and Reagents. All chemicals and solvents (95% purity or higher) were used as received from various commercial suppliers. Further descriptions of chemical sources and stock solutions are provided in SI Text-1.

Reaction Kinetics. Kinetic studies of Fe(VI) reactions were performed in a batch reactor in the pH range 6.0−9.5. Preliminary experiments showed that the reaction of Fe(VI) with all β-lactams and the model compounds investigated in this study followed first-order with respect to Fe(VI) and the organic compounds (SI Text-2). The apparent second-order reaction rate constants were therefore determined using pseudo-first-order kinetic conditions with either Fe(VI) or the compound in excess. Fe(VI) concentrations were measured with the ABTS method.[55] β-lactam concentrations were analyzed by HPLC/UV (SI Text-3).

Oxidation of β-Lactams in a Wastewater Effluent Matrix. Secondary wastewater effluents spiked with each β-lactam (2 μM) were treated with a range of Fe(VI) doses (0−100 μM) at pH 7.0 and 8.5, buffered with 20 mM carbonate and 10 mM borate, respectively. The Fe(VI) consumption kinetics in the wastewater effluents were negligibly affected by the presence of each β-lactam at 2 μM (data not shown). Therefore, the obtained elimination data using 2 μM of β-lactam could be used to simulate the elimination behavior of β-lactams at trace levels (i.e., sub μg L^{-1}). The wastewater was taken from the effluent of a conventional activated sludge process at a municipal wastewater treatment plant in Gwangju, Korea (GJWW, DOC = 7.3 mgC/L). Concentrations of β-lactam and Fe(VI) were determined during Fe(VI) treatment (0−180 min) and after complete consumption of Fe(VI) (SI Text-4).

Identification of Transformation Products. Twenty μM of PENG and CEX was prepared separately in pH 7 phosphate buffered solution (1 mM) and treated with a range of Fe(VI) doses (10−120 μM). Additional sets of PENG and CEX samples prepared under the same conditions were treated with ozone (10−60 μM) in the presence of the $^{\bullet}$OH scavenger *tert*-butanol (10 mM),[56] in order to generate sulfoxide transformation products for use as confirmatory analytical standards.[52] The reaction mixtures were left for 3 h to allow

completion of the oxidation reactions. Samples were filtered through 0.45 μm PVDF filters and kept at 4 °C until analysis using an HPLC (Alliance 2695, Waters, Milford, MA, U.S.A.) coupled with a triple-quadrupole tandem mass spectrometer (Micromass, Waters, Manchester, U.K.) (SI Text-8). The CEX samples treated with Fe(VI) were also analyzed for ammonia using the phenate method.[57]

Antibacterial Activity of β-Lactam Transformation Product Mixtures. Transformation product mixtures were obtained for the β-lactams PENG, CLOX, AMX, and CEX by reacting 10 μM β-lactam with Fe(VI) (0−60 μM) at pH 7 (1 mM phosphate buffer). Residual β-lactam concentrations after completion of the reaction were determined by HPLC/UV. The antibacterial activities of the reaction solutions were determined by broth microdilution assay.[51] This assay measures growth inhibition of *B. subtilis* (KCCM, 11316) as a reference bacterial strain inoculated to serially diluted samples prepared from each reaction solution. The growth inhibition values (%) measured for each member of the dilution series derived from each Fe(VI)-treated β-lactam sample were then plotted as a function of the logarithmic sample dilution factors to yield dose−response relationships. Each dose−response curve was fitted to a symmetric logistic function by optimizing for relative dilution factors (RDF) causing a 50% growth inhibition (EC_{50}). The antibacterial activity of samples, expressed as a "potency equivalent" (PEQ) value, was calculated as the ratio of the EC_{50} of the untreated β-lactam (10 μM) to the EC_{50} of each treated sample, i.e., PEQ = EC_{50}(β-lactam)/EC_{50}(sample). Additional details on the microdilution assay and the corresponding data evaluation are provided in SI Text-9.

RESULTS AND DISCUSSION

Reaction Kinetics with β-Lactam Antibiotics and Model Compounds. The kinetics for reactions of Fe(VI) with all selected compounds followed a second-order rate law (SI Text-2). The apparent second-order rate constants (in M^{-1} s^{-1}) were determined in the pH range 6.0−9.5 by measuring Fe(VI) decrease in the presence of excess organic compound (k_{app}) or by measuring organic compound decrease in the presence of excess ferrate (k'_{app}). The k_{app} differs from the k'_{app} by a stoichiometric factor η, which defines the number of Fe(VI) molecules consumed per molecule of target compound under the experimental conditions. Figure 1 depicts the measured values of k_{app} (filled circles) and k'_{app} (empty triangles) at varying pH. The measured k_{app} and k'_{app} values were quite similar and the k_{app}/k'_{app} was 1.0(±0.3), 1.1(±0.4), 1.2(±0.4), and 1.3(±0.4) for PENG, CLOX, AMX, and CEX, respectively, indicating that the stoichiometry for reaction of Fe(VI) with the selected β-lactams is close to one (i.e, η = 1). The similar k_{app} and k'_{app} values also indicate that the rate constants measured by decrease of Fe(VI) (k_{app}), which has been the typical practice in previous Fe(VI) studies, can be used to predict the transformation kinetics of trace levels of β-lactams during water treatment with excess Fe(VI). At pH 7, k_{app} values for the β-lactams were: AMX (771 M^{-1} s^{-1}) > CEX (686 M^{-1} s^{-1}) > AMP (418 M^{-1} s^{-1}) > CLOX (116 M^{-1} s^{-1}) ≈ PENG (114 M^{-1} s^{-1}).

For PENG, CLOX, and CEX, k_{app} decreased with increasing pH, while for AMP, APA, and AMX, it increased with increasing pH in the pH range 6−7 and then decreased in the pH range 7−9.5 (Figure 1). The observed pH-dependent variations in k_{app} could be explained by considering species-specific reactions between Fe(VI) species ($HFeO_4^- \rightleftharpoons FeO_4^{2-}$ + H^+, $pK_{a,HFeO_4^-}$ = 7.2[58]) and acid−base species of an ionizable substrate ($SH^+ \rightleftharpoons S + H^+$ or $SH \rightleftharpoons S^- + H^+$, $pK_{a,SH}$, where S refers to an amine- or phenol-moiety for the selected β-lactam). Based on this kinetic model, eq 1 applies for the loss of Fe(VI) or substrate,

$$\frac{d[Fe(VI)]_{tot}}{dt} = \eta\frac{d[S]_{tot}}{dt} = -k_{app}[Fe(VI)]_{tot}[S]_{tot} = -\sum_{i,j}^{n,m} k_{i,j}\alpha_i\beta_j[Fe(VI)]_{tot}[S]_{tot} \quad (1)$$

where $[Fe(VI)]_{tot}$ and $[S]_{tot}$ represent the total concentration of Fe(VI) and substrate, respectively, η represents the stoichiometric factor (with $k_{app} = \eta k'_{app}$), $k_{i,j}$ is the species-specific second-order rate constant between Fe(VI) and substrate species, and α_i and β_j represent the equilibrium distribution coefficients of Fe(VI) and substrate species. The species-specific second-order rate constants ($k_{i,j}$) were in turn calculated from least-squares nonlinear regressions of experimental k_{app} (or k'_{app}) according to eq 2, using GraphPad Prism (www.graphpad.com).

$$k_{app} = \sum_{i,j}^{n,m} k_{i,j}\alpha_i\beta_j \quad (2)$$

The $k_{i,j}$ values determined for the selected β-lactams and model compounds are summarized in Table S1. In all cases, eq 2 could explain the experimental k_{app} well ($R^2 \geq 0.94$). In Figure 1, the solid lines represent the model calculations for k_{app} and the dashed or dotted lines represent the calculated apparent species-specific reaction rate constants as a function of pH.

Penicillin G (PENG, Figure 1a) and cloxacillin (CLOX, Figure 1b). The pH-dependent k_{app} for PENG and CLOX was almost identical and decreased from 150−156 to 10−16 M^{-1} s^{-1} with increasing pH from 6.0 to 9.5. The structure of CLOX differs from PENG by the presence of a chlorine atom on the benzene ring and the isoxazole moiety (Table S1). The same k_{app} for PENG and CLOX indicates that the thioether is the main reaction site for both compounds and the chloro-benzene and isoxazole moieties are nonreactive to Fe(VI) compared to the thioether. This was also supported by a low second-order rate constant for the reaction of Fe(VI) with 3,5-dimethyl-isoxazole (i.e., k_{app} = 0.2 M^{-1} s^{-1} at pH 8, Table S1) as a substructure model compound. The pH-dependence of k_{app} could be explained by eq 3, which considers the reactions of $HFeO_4^-$ ($k_{HFeO_4^-/S}$= 1.8 ± (0.2) × 10^2 M^{-1} s^{-1} for PENG and =1.8 ± (0.3) × 10^2 M^{-1} s^{-1} for CLOX) and FeO_4^{2-} ($k_{FeO_4^{2-}/S}$ = 9.7 ± (1.8) M^{-1} s^{-1} for PENG and =15.4 ± (4.0) M^{-1} s^{-1} for CLOX) with the thioether of PENG or CLOX. The higher reactivity of $HFeO_4^-$ compared to FeO_4^{2-} has been observed in many previous studies.[20−36]

$$k_{app-PENG}(\text{or } k_{app\text{-}CLOX}) = k_{HFeO_4^-/S}\alpha_{HFeO_4^-} + k_{FeO_4^{2-}/S}\alpha_{FeO_4^{2-}} \quad (3)$$

Ampicillin (AMP, Figure 1c), 2-amino-2-phenylacetamide (APA, Figure 1d), and amoxicillin (AMX, Figure 1e). The observed higher k_{app} of AMP compared to PENG or CLOX can be attributed to the presence of an amine group in

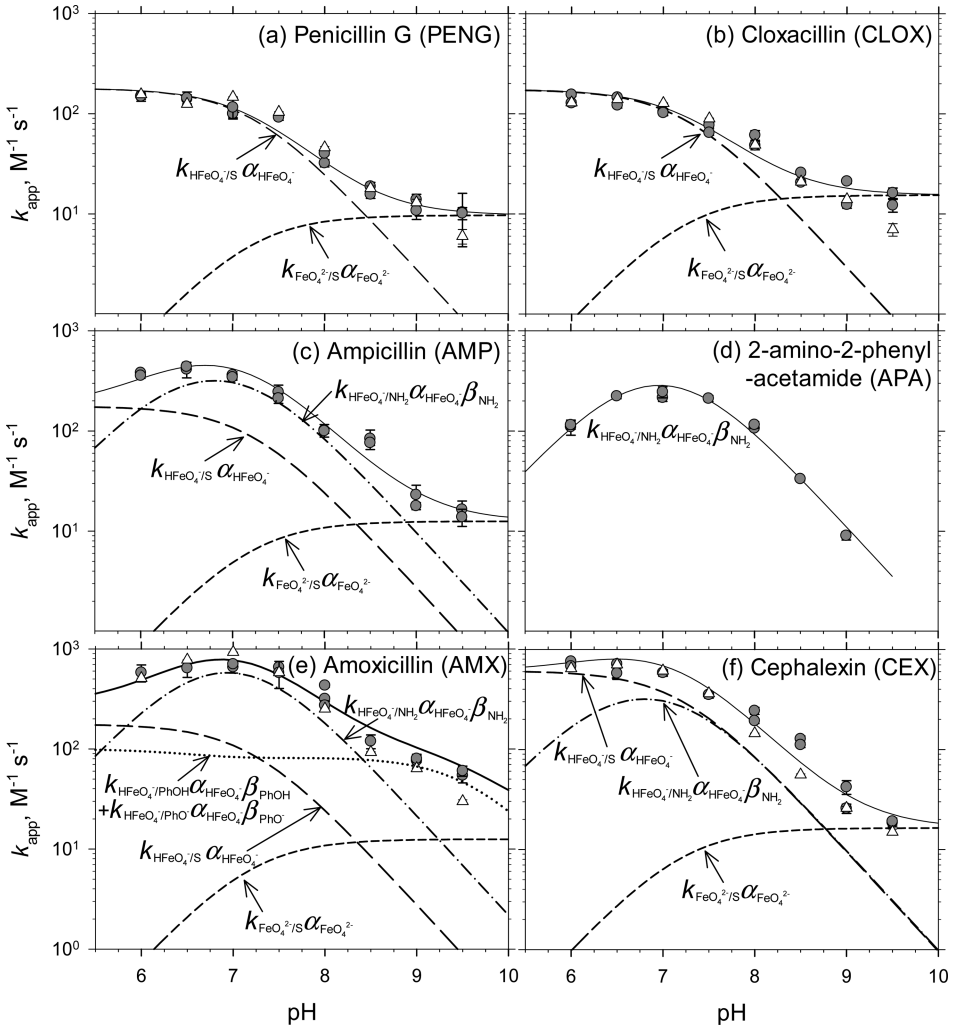


Figure 1. Apparent second-order rate constants (k_{app}) for the reaction of Fe(VI) with selected β-lactam antibiotics and model compounds as a function of pH: (a) penicillin G (PENG), (b) cloxacillin (CLOX), (c) ampicillin (AMP), (d) 2-amino-2-phenylacetamide (APA), (e) amoxicillin (AMX), and (f) cephalexin (CEX). Filled circles represent k_{app} determined from the decrease of Fe(VI) in the presence of excess target compounds and empty triangles represent k'_{app} determined from the decrease of a target compound in the presence of excess Fe(VI). The solid lines represent the model calculations for k_{app}. The dashed or dotted lines represent the calculated species-specific rate constants for the reaction of $HFeO_4^-$ with thioether ($k_{HFeO_4^-/S}\ \alpha_{HFeO_4^-}$, long dashed), deprotonated amine ($k_{HFeO_4^-/NH_2}\ \alpha_{HFeO_4^-}\ \beta_{NH_2}$, long–short dashed), and phenol ($k_{HFeO_4^-/PhOH}\ \alpha_{HFeO_4^-}\ \beta_{PhOH}$ + $k_{HFeO_4^-/PhO^-}\ \alpha_{HFeO_4^-}\ \beta_{PhO^-}$, dotted), and the reaction of FeO_4^{2-} with thioether ($k_{FeO_4^{2-}/S}\ \alpha_{FeO_4^{2-}}$, short dashed).

AMP in addition to the thioether. Accordingly, the pH-dependence of $k_{app\text{-}AMP}$ was explained by eq 4,

$$k_{app-AMP} = k_{HFeO_4^-/S}\alpha_{HFeO_4^-} + k_{FeO_4^{2-}/S}\alpha_{FeO_4^{2-}} + k_{HFeO_4^-/NH_2}\alpha_{HFeO_4^-}\beta_{NH_2} \quad (4)$$

in which the reaction of $HFeO_4^-$ with deprotonated amine (i.e.,$k_{HFeO_4^-/NH_2}\ \alpha_{HFeO_4^-}\ \beta_{NH_2}$) is additionally included compared to eq 3. The reaction with protonated amine was not considered because protonated amines typically show negligible reactivity to oxidants.[59] In addition, $k_{HFeO_4^-/S}$ and $k_{FeO_4^{2-}/S}$ values of 1.8×10^2 M^{-1} s^{-1} and 12.6 M^{-1} s^{-1}, respectively, were used for the regression with eq 4. These are the average rate constants determined for PENG and CLOX. This approach is reasonable as the three β-lactams (PENG, CLOX, and AMP) contain almost identical thioether moieties.

Regression trials taking into account the amine speciation yielded the best fit when a pK_a of 6.4 (±0.5) was used for the amine moiety. This is lower than the pK_a of 6.7 predicted by SPARC (https://archemcalc.com), or 7.2 reported in literature.[60] It should be noted, however, that large variability exists in pK_a measurements reported for this functional group in the literature.[61] Therefore, the pK_a of the amines obtained from fitting with the kinetic data were used in this study. On the basis of the best fitting pK_a of 6.4, the $k_{HFeO_4^-/NH_2}$was determined to be $6.2 \pm (2.0) \times 10^2$ M^{-1} s^{-1}.

The bell-shaped profile of the pH-dependent k_{app} for APA, a model compound for amine-moiety of AMP, could be expained by considering the reaction of $HFeO_4^-$ with the deprotonated amine (eq 5).

$$k_{app\text{-}APA} = k_{HFeO_4^-/NH_2}\alpha_{HFeO_4^-}\beta_{NH_2} \quad (5)$$

The regression of $k_{app\text{-}APA}$ with eq 5 yielded a pK_a of 6.7 for the amine (compared to 7.2 predicted by SPARC) and $k_{HFeO_4^-/NH_2}$of 7.1 ± (1.2) × 10^2 M^{-1} s^{-1}. These pK_a and $k_{HFeO_4^-/NH_2}$ values are comparable to those for AMP, which is

dx.doi.org/10.1021/es5028426 | Environ. Sci. Technol. 2014, 48, 10380−10389

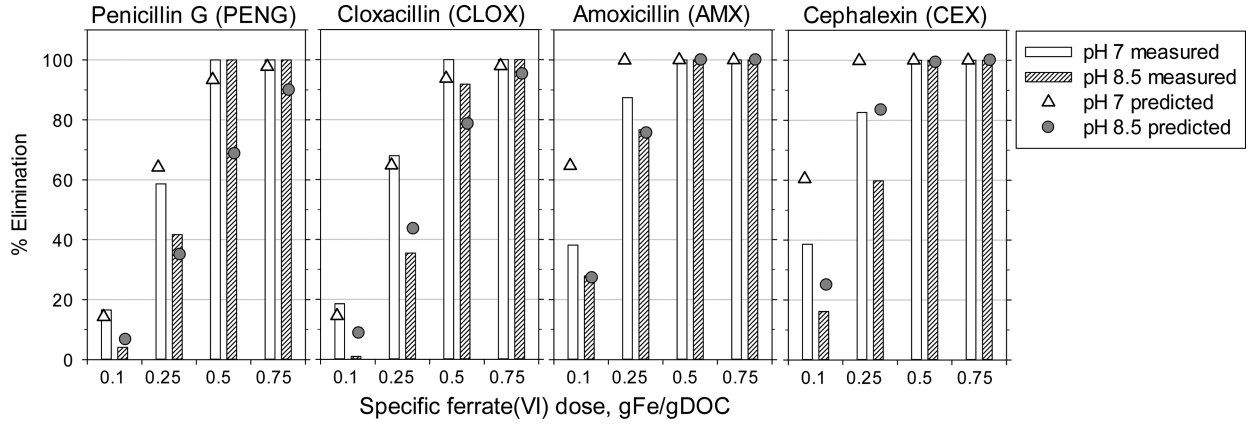


Figure 2. Oxidative elimination of β-lactams (PENG, CLOX, AMX, and CEX) spiked at 2 μM in a wastewater effluent (DOC = 7.3 mgC/L) at pH 7 and 8.5 as a function of Fe(VI) dose, gFe/gDOC = 0.1 (13 μM), 0.25 (33 μM), 0.5 (66 μM), and 0.75 (100 μM), where the values in parentheses indicate absolute Fe(VI) dose in molar-scale. The bars represent the measured data and the symbols (triangles and circles) represent the model predictions. Residual β-lactam concentrations were measured after 1 h.

consistent with the similar structure of the amine moiety of these two compounds.

The observed larger k_{app} of AMX compared to AMP is attributed to the presence of the additional phenolic moiety. Accordingly, the pH-dependence of $k_{app\text{-}AMX}$ was explained by eq 6 in which the reaction of $HFeO_4^-$ with protonated and deprotonated phenolic moiety is additionally included compared to eq 4.

$$k_{app\text{-}AMX} = k_{HFeO_4^-/S}\alpha_{HFeO_4^-} + k_{FeO_4^{2-}/S}\alpha_{FeO_4^{2-}} + k_{HFeO_4^-/NH_2}\alpha_{HFeO_4^-}\beta_{NH_2} + k_{HFeO_4^-/PhOH}\alpha_{HFeO_4^-}\beta_{PhOH} + k_{HFeO_4^-/PhO^-}\alpha_{HFeO_4^-}\beta_{PhO^-} \quad (6)$$

For regressions with eq 6, $k_{HFeO_4^-/S}$ and $k_{FeO_4^{2-}/S}$ values of 1.8 $\times$ 10^2 M^{-1} s^{-1} and 12.6 M^{-1} s^{-1} were again used based on the same thioether moiety for these compounds. In addition, a $k_{HFeO_4^-/PhOH}$ of 1.0 $\times$ 10^2 M^{-1} s^{-1} and $k_{HFeO_4^-/PhO^-}$ of 2.1 $\times$ 10^4 M^{-1} s^{-1} were used, both of which could be estimated based on the known reactivity of $HFeO_4^-$ to protonated and deprotonated phenol.[28,62] The $k_{HFeO_4^-/PhOH}$ and $k_{HFeO_4^-/PhO^-}$ values had to be estimated because these could not be determined accurately from the regression with eq 6 due to the relatively low contribution of Fe(VI)/phenol reaction to the overall reaction rate. As a result of the regression, a pK_a of 6.7 for the amine moiety (compared to 6.9 predicted by SPARC) and $k_{HFeO_4^-/NH_2}$ of 1.4 $\pm$ (0.5) $\times$ 10^3 M^{-1} s^{-1} were obtained. This $k_{HFeO_4^-/NH_2}$ for AMX is ~2-fold higher than that of AMP ($k_{HFeO_4^-/NH_2}$= 6.2 $\times$ 10^2 M^{-1} s^{-1}).

Cephalexin (CEX, Figure 1f). CEX showed higher k_{app} than AMP. As CEX and AMP contain nearly the same amine moiety, the difference in reactivity of these two compounds could be attributed to the different thioether structures or the presence of the olefin moiety in CEX. Kinetic experiments with 3-methylcrotonic acid as a structural model compound for the olefin moiety yielded a second-order rate constant of 3.5 M^{-1} s^{-1} for the reaction with $HFeO_4^-$ (SI Table S1). This indicates that the olefin moiety of CEX is not responsible for the observed larger reactivity of CEX. Alternatively, the higher reactivity of CEX could be explained by decreased steric hindrance toward reaction of Fe(VI) with the thioether in the six-membered ring system—which lacks an adjacent dimethyl group—compared to the thioether in the five-membered ring systems of the penicillins. Additional discussions on the comparison of Fe(VI) reactivity toward thioether moieties is provided in SI Text-5.

The pH-dependent $k_{app\text{-}CEX}$ data were analyzed with eq 7, assuming a $k_{HFeO_4^-/NH_2}$ of 6.2 $\times$ 10^2 M^{-1} s^{-1} and a pK_a of 6.4 for the amine moiety. The latter two values were taken from the data obtained for AMP.

$$k_{app\text{-}CEX} = k_{HFeO_4^-/S}\alpha_{HFeO_4^-} + k_{FeO_4^{2-}/S}\alpha_{FeO_4^{2-}} + k_{HFeO_4^-/NH_2}\alpha_{HFeO_4^-}\beta_{NH_2} \quad (7)$$

The regression with eq 7 yielded $k_{HFeO_4^-/S}$ = 6.1 $\pm$ (2.0) $\times$ 10^2 M^{-1} s^{-1} and $k_{FeO_4^{2-}/S}$= 16.4 $\pm$ (8.7) M^{-1} s^{-1} for the reaction of $HFeO_4^-$ and FeO_4^{2-} with the thioether of CEX, respectively.

Additional discussions are provided in SI with respect to the comparison of our k_{app} data with literature values (SI Text-6) and the prediction of k_{app} for other penicillins and cephalosporins (SI Text-7).

β-Lactam Elimination in a Wastewater Effluent Matrix. The significant reactivity of β-lactams toward Fe(VI) (e.g., k_{app} = 110−770 M^{-1} s^{-1} for pH 7) indicates that these compounds can likely be effectively eliminated during wastewater effluent treatment with Fe(VI). To confirm this, experiments for the elimination of selected β-lactams (i.e., PENG, CLOX, AMX, and CEX) were performed in a real wastewater effluent. Figure 2 shows the elimination of individually spiked β-lactams at a concentration of 2 μM in a wastewater (GJWW, DOC = 7.3 mgC L^{-1}) at pH 7 and 8.5. Fe(VI) doses were 13, 33, 66, and 100 μM, which corresponded to specific Fe(VI) doses (i.e., mass-based Fe(VI) to dissolved organic carbon ratios) of 0.1, 0.25, 0.50, and 0.75 gFe/gDOC, respectively. All β-lactams except CLOX were ≥98% transformed at a specific Fe(VI) dose of ≥0.5 (= 3.7 mgFe L^{-1}), confirming their efficient elimination in a real effluent matrix. Elimination of CLOX was slightly less for pH 8.5 compared to the other β-lactams (e.g., 93% elimination was obtained at a specific Fe(VI) dose of 0.5).

As a next step, the elimination of each β-lactam (S) in the wastewater effluent was predicted using the measured apparent second-order rate constants (k_{app}, SI Table S1) and Fe(VI)

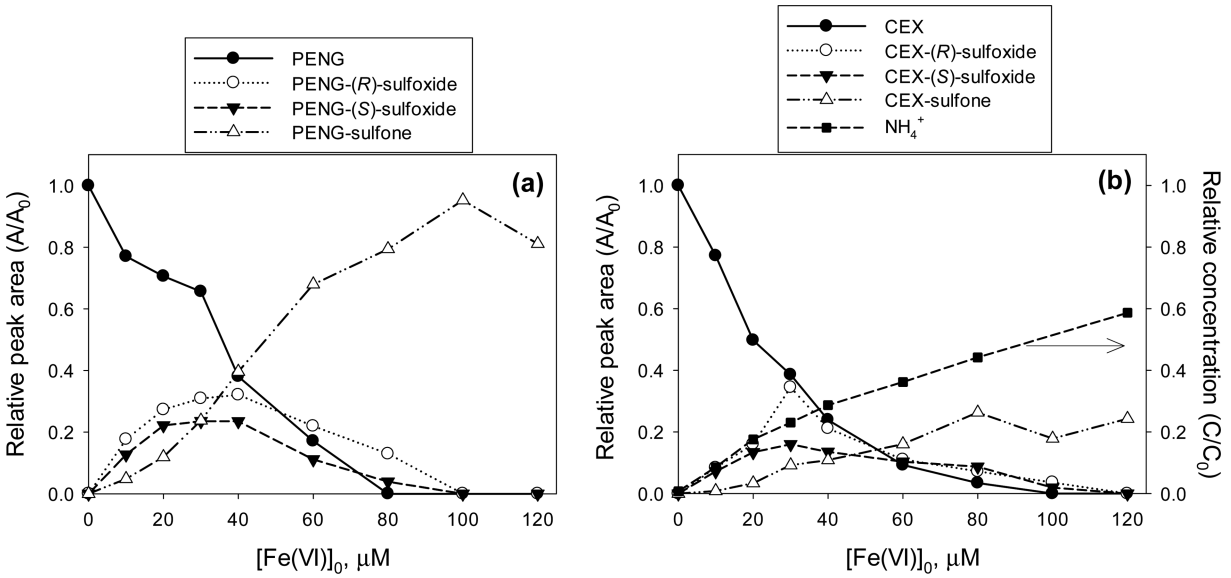


Figure 3. Changes of the relative peak areas (A/A_0) for (a) PENG and its transformation products, and (b) CEX and its transformation products, for reactions of $[PENG]_0$ or $[CEX]_0$ = 20 μM with $[Fe(VI)]_0$ = 0−120 μM at pH 7 (1 mM phosphate buffer). Relative peak areas for sulfoxide products were adjusted as described in SI Text-8 while relative peak areas of the parent β-lactams and β-lactam-sulfones were normalized by the peak area corresponding to an initial 20 μM concentration of the respective parent β-lactam. Ammonia (NH_4^+) concentrations were normalized by the initial concentration of CEX (i.e., 20 μM).

exposures, according to eq 8, which can be derived by integration of eq 1 (for η = 1, as noted above),

$$\frac{[S]_\tau}{[S]_0} = \exp[-k_{app}\int_0^\tau [Fe(VI)]dt] \quad (8)$$

where $\int_0^\tau [Fe(VI)]dt$ represents the Fe(VI) exposure.

SI Figures S4 and S5 show the decrease in Fe(VI) concentration in GJWW effluent for various Fe(VI) doses at pH 7 and 8.5, respectively. The decrease in Fe(VI) concentration was faster at pH 7 (<40 min) than pH 8.5 (>60 min). This is consistent with the presence of more $HFeO_4^-$ with decreasing pH and the higher reactivity of $HFeO_4^-$ compared to FeO_4^{2-} with respect to its reaction with the effluent organic matter or to Fe(VI) self-decay.[63] Accordingly, the Fe(VI) exposures for pH 7 (1.3−31 mg L^{-1} min) were lower than those for pH 8.5 (3.6−154 mg L^{-1} min) by a factor of 3 at the same Fe(VI) dose (SI Figure S6). Despite the lower Fe(VI) exposures, elimination levels of the four β-lactams were higher for pH 7 than pH 8.5 (Figure 2). This can be explained by the 5−9 fold larger k_{app} values for pH 7 compared to pH 8.5 (Figure 1, SI Table S1). As shown in Figure 2, the measured and predicted % eliminations of β-lactams were reasonably consistent.

Transformation Products and Pathways. *PENG.* Three major products with transformations at the thioether moiety were found from the reaction of Fe(VI) with PENG in HPLC/MS analyses. Two peaks with m/z of 351 in full-scan positive-mode ESI ($M + H^+$) were detected at retention times (RT) of 5.3 and 6.5 min, respectively (SI Figures S8 and S11). These two peaks—each with a mass of an additional oxygen atom (M = 16) compared to PENG—were consistent with the stereoisomeric PENG-(R)- and PENG-(S)-sulfoxides, which have also previously been observed as the primary products in reaction of ozone with PENG.[52] The identities of these products were confirmed by comparison with a standard mixture prepared by treatment of PENG with ozone (SI Text-8). In a previous study,[52] PENG-(R)-sulfoxide was found to elute earlier than PENG-(S)-sulfoxide when using a C16 reversed-phased HPLC column. Therefore, the two peaks observed here at RTs of 5.3 and 6.5 min were assigned to PENG-(R)- and PENG-(S)-sulfoxide, respectively. An additional peak with m/z of 367 ($M + H^+$) was detected at RT of 12.5 min for the Fe(VI)-treated samples (SI Figures S8 and S11), but was not observed in ozone-treated samples (SI Figure S9), consistent with prior work.[52] This peak—with a mass of additional two oxygen atoms (M = 32) compared to PENG—was assigned to PENG-sulfone.

Figure 3a shows the changes of the relative peak areas (A/A_0) for PENG and its transformation products (i.e., PENG-(R)-sulfoxide, PENG-(S)-sulfoxide, and PENG-sulfone) for reactions of 20 μM of PENG with a range of initial Fe(VI) concentrations ($[Fe(VI)]_0$). The peak areas of PENG-(R)- and PENG-(S)-sulfoxides increased for $[Fe(VI)]_0 \leq 30$ μM and then decreased with further increasing $[Fe(VI)]_0$. PENG-(R)-sulfone formation peaked after PENG-(R)- and PENG-(S)-sulfoxide formation and then continued to increase with increasing $[Fe(VI)]_0$. The yields of PENG-(R)- and PENG-(S)-sulfoxide from the reaction of Fe(VI) with PENG were estimated to be 55% and 45%, respectively, based on the initial increases of relative peak areas for each product, which were adjusted for differences in PENG and PENG-sulfoxide absorbances as discussed in SI Text-8. The peak areas of PENG-sulfone were adjusted by scaling to the peak area for PENG at an initial concentration of 20 μM. The peak evolution patterns in Figure 3a are consistent with initial transformation of PENG by Fe(VI) to PENG-(R)- and PENG-(S)-sulfoxide followed by further transformation to PENG-sulfone. Considering the generally lower reactivity of Fe(VI) toward organic compounds compared to ozone,[59,62] the observed susceptibility of PENG-sulfoxide to further oxidation by Fe(VI) is unexpected. SI Scheme S1 summarizes the proposed transformation pathway of PENG during Fe(VI) oxidation.

CEX. The transformation products resulting from reaction of Fe(VI) at the thioether moiety of CEX were comparable to

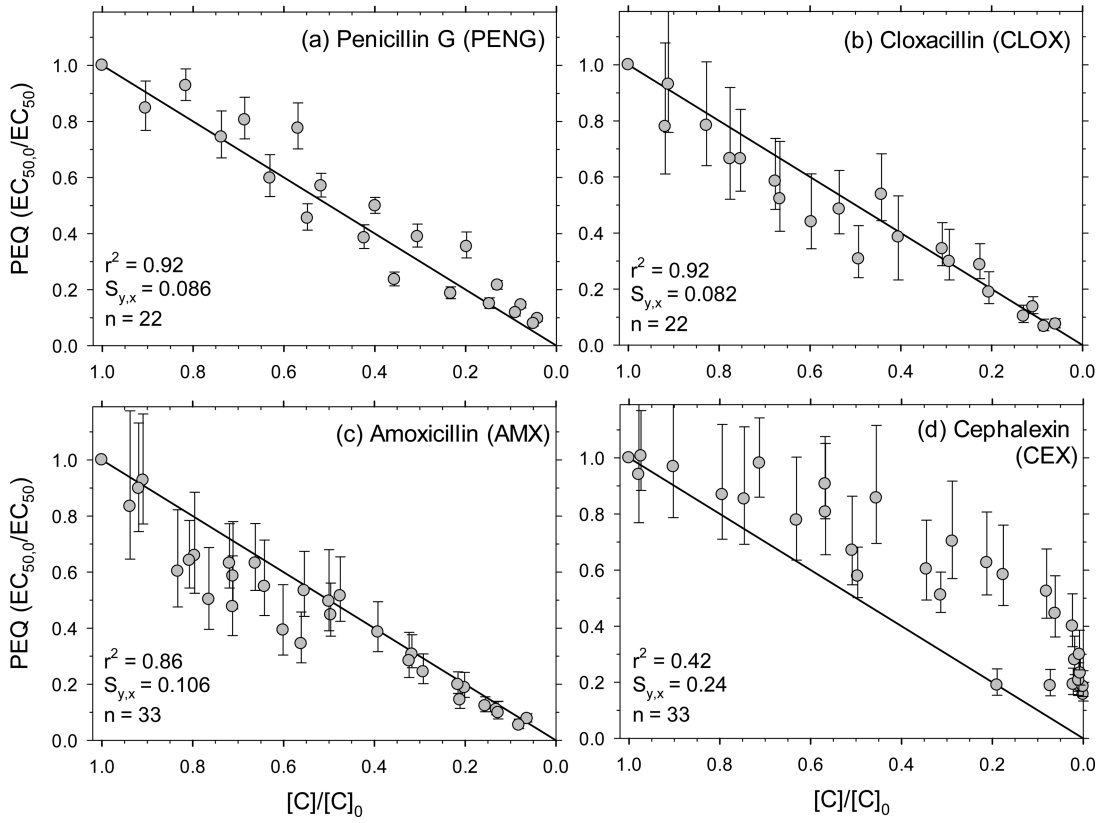


Figure 4. Plots of the PEQ vs the relative β-lactam concentration ($[C]/[C]_0$) as deactivation stoichiometry for oxidation of (a) penicillin G (PENG), (b) cloxacillin (CLOX), (c) amoxicillin (AMX), and (d) cephalexin (CEX). Experimental conditions: $[\beta\text{-lactam}]_0$ = 10 μM, $[Fe(VI)]_0$ = 0−60 μM, and pH = 7 (1 mM phosphate buffer). The error bars depict 95% confidence limits for the fitting of each dose−response measurement. The r^2 is the coefficient of determination for the regression of the data with an equation $y = x$. The $S_{y,x}$ is $(SS/df)^{1/2}$ where SS is the sum-of-squares of the distance of the regression from the data points and df is the degrees of freedom of the fit (i.e., n = number of data points).

those of PENG. In HPLC/MS analyses, two peaks with m/z of 364 ($M + H^+$) were detected at RT of 2.3 and 2.9 min, respectively (SI Figures S14 and S17). These two peaks—each with a mass of an additional oxygen atom (M = 16) compared to CEX—were assigned to the stereoisomeric CEX-(R)- and CEX-(S)-sulfoxides, respectively, based on comparison with a standard mixture prepared by treatment of CEX with ozone and previous observations pertaining to sulfoxide elution order (SI Text-8).[52] An additional peak with m/z of 380 ($M+H^+$) was detected at RT of 3.2 min for the Fe(VI)-treated samples (SI Figures S14 and S17), but was not observed in ozone-treated samples (SI Figure S15), consistent with prior work.[52] This peak—with a mass of two additional oxygen atoms (M = 32) compared to CEX—was assigned to CEX-sulfone..

Figure 3b shows the changes of relative peak areas (A/A_0) for CEX and its transformation products (i.e., CEX-(R)-sulfoxide, CEX-(S)-sulfoxide, and CEX-sulfone) for reactions of 20 μM of CEX with a range of $[Fe(VI)]_0$. The relative peak areas of CEX-(R)- and CEX-(S)-sulfoxide increased and then decreased with increasing $[Fe(VI)]_0$. CEX-(R)-sulfone formation peaked after CEX-(R)- and CEX-(S)-sulfoxide formation and continued to increase with $[Fe(VI)]_0$. The yields of CEX-(R)- and CEX-(S)-sulfoxide from the reaction of Fe(VI) with CEX were estimated to be ∼40% and ∼30%, respectively, based on the initial increases of relative peak areas for each product, which were adjusted for differences in CEX and CEX-sulfoxide absorbances as discussed in SI Text-8. The peak areas of CEX-sulfone were adjusted by scaling to the peak area for CEX at an initial concentration of 20 μM. The evolution patterns of the transformation products indicate that CEX is transformed to CEX-(R)- and CEX-(S)-sulfoxides as primary products and then further transformed to CEX-sulfone.

Compared to PENG, the relative peak areas for CEX products with a transformed thioether moiety are lower. This can be explained by the fact that Fe(VI) also reacts with the amine moiety of CEX and its thioether-transformed products. At pH 7, the reaction rate of Fe(VI) with the amine (k_{app} = 305 M^{-1} s^{-1}) is comparable to that of the thioether (k_{app} = 374 M^{-1} s^{-1}) (Figure 1). Fe(VI) reaction with the olefin moiety of CEX is expected to be minimal due to the low reactivity of Fe(VI) toward the olefin moiety of MCA (k_{app} = 2.3 M^{-1} s^{-1} for pH 7).

Ammonia as ammonium ion (NH_4^+) was also formed in the Fe(VI)-CEX reaction, with increasing concentration as the $[Fe(VI)]_0$ was increased (Figure 3b). The molar yield of ammonia (i.e., $[NH_4^+]/[CEX]_0$) was ∼60% for the condition of $[Fe(VI)]_0$ = 120 μM, at which CEX was completely transformed. The missing nitrogen balance (i.e., ∼40%) can be partly explained by the formation of CEX-sulfone containing the intact amine-moiety. On the basis of adjusted relative peak area (A/A_0), CEX-sulfone is estimated to account for ∼25% of the nitrogen mass balance. Therefore, C—N bond cleavage and ammonia formation must represent the major reaction pathway for the reaction of Fe(VI) with the amine-moiety of CEX. Similar C—N bond cleavage and the consequent formation of carbonyl or ammonia have been observed for the reaction of Fe(VI) with primary aliphatic amines[35,64,65] or amino acids.[66] SI Scheme S2 shows the proposed reaction mechanism for oxidation of the amine-moiety of CEX by Fe(VI). According to

this mechanism, products with a diacetyl moiety are expected to form (see SI Figure S20). However, no compounds with masses corresponding to the anticipated diacetyl products could be detected in either postive- or negative-mode full-scan HPLC/MS analyses, suggesting that diacetyl products may have been further transformed via hydrolysis or escaped MS detection due to poor retention and/or low method sensitivity. SI Scheme S3 summarizes the proposed transformation pathways of CEX during Fe(VI) oxidation. The predicted transformation pathways of CLOX, AMP, and AMX are also discussed in SI Text-8.

Antibacterial Activity of Transformation Product Mixtures. Figure 4 shows the decrease of PEQ, a quantitative measure of antibacterial activity, as a function of the relative β-lactam concentration, $[C]/[C]_0$, after Fe(VI) oxidation of the β-lactams PENG, CLOX, AMX, and CEX. The lines in Figure 4 represent an ideal one-to-one deactivation stoichiometry (i.e, PEQ = $[C]/[C]_0$) in which the transformation products contain negligible antibacterial activity compared to the parent β-lactam and consumption of one mole fraction of parent β-lactam therefore results in a loss of one PEQ unit. If some of the transformation products were to retain appreciable antibacterial activity compared to the parent β-lactam, then the measured PEQ would deviate positively from the line of ideal stoichiometry. In contrast, negative deviations from the line would suggest inhibition of a given parent β-lactam's activity by transformation products.

For PENG, CLOX, and AMX, the decrease of PEQ closely followed the line of one-to-one stoichiometry (Figure 4a−c). In the intermediate transformation range (e.g., $[C]/[C]_0$ = 0.2−0.8), PENG showed some positive deviations while CLOX and AMX showed apparent negative deviations. Nevertheless, for more than 80% transformation of the parent compound ($[C]/[C]_0 \leq 0.2$), the decrease of PEQ followed the line closely. This indicates that the transformation products of PENG, CLOX, and AMX contain significantly lower antibacterial activity compared to each parent compound. The average antibacterial activity of the transformation products mixture compared to the parent compound was estimated to be 5(±3)%, 0(±3)%, and −2(±2)% for PENG, CLOX, and AMX, respectively, at the condition of $[C]/[C]_0 \leq 0.2$ (SI-Text-9 for details). PENG-(*R*)-sulfoxide was previously determined to be 15% as active as PG using the bioassay similar to this work.[52] Nevertheless, it should be noted that during exposure to Fe(VI), PENG-(*R*)-sulfoxide is not stable and further transformed to PENG-sulfone, which is also expected to have significantly lower antibacterial activity than PENG itself.[67,68]

In contrast, CEX showed significant positive deviations of PEQ from the ideal stoichiometric line (Figure 4d). For more than 80% transformation of CEX ($[C]/[C]_0 < 0.2$), the residual PEQ was 0.26 ± 0.11 (0.16−0.40). Thus, some of the transformation products of CEX retain significant antibacterial activity relative to CEX itself (≥26%). CEX-(*R*)-sulfoxide was previously determined to be 83% as active as CEX using a similar bioassay as applied here.[52] However, CEX-(*R*)-sulfoxide alone does not fully explain the residual antibacterial activity as it is further transformed at larger Fe(VI) exposure. On the basis of observed product evolution patterns and their structure, CEX-sulfone is a probable candidate for the observed residual activity. This would be consistent with observations that β-lactam sulfones—while significantly less active than the parent β-lactams from which they are derived—can exhibit activities on the order of 1/10 of the parent β-lactams.[67,68] The PEQ of CEX-sulfone could not be accurately estimated in this study due to the presence of unidentified CEX transformation products and the uncertainty in the estimated CEX-sulfone concentration by relative peak areas. Despite the formation of products with measurable residual activity, the data reported here indicate that Fe(VI) oxidation of CEX at a typical treatment condition (e.g., $[Fe(VI)]_0 \leq 200\ \mu M$) can be expected to lead to ∼80% reduction of the antibacterial activity induced by CEX.

■ ASSOCIATED CONTENT

Supporting Information

Nine texts, 3 tables, 21 figures, and 3 schemes addressing materials, experimental procedures, and additional data are included in the Supporting Information. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This study was funded by the General Researcher Program (NRF-2012R1A1A1010985) and the Mid-Career Researcher Program (NRF-2013R1A2A2A03068929) through the National Research Foundation of Korea funded by the Ministry of Science ICT & Future Planning. G.A.D.D.V. was supported by the GIST global internship program. We thank S. Kang for assistance with LC/MS analysis and Prof. H.-G. Hur for assistance with antibacterial assay.

■ REFERENCES

(1) Ternes, T. A.; Joss, A. *Human Pharmaceuticals, Hormones and Fragrances. The Challenge of Micropollutants in Urban Water Management*; IWA Publishing: London, 2006.

(2) Sumpter, J. P.; Johnson, A. C. 10th anniversary perspective: Reflections on endocrine disruption in the aquatic environment: From known knowns to unknown unknowns (and many things in between). *J. Environ. Monit.* **2008**, *10*, 1476−1485.

(3) Michael, I.; Rizzo, L.; McArdell, C. S.; Manaia, C. M.; Merlin, C.; Schwartz, T.; Dagot, C.; Fatta-Kassinos, D. Urban wastewater treatment plants as hotspots for the release of antibiotics in the environment: A review. *Water Res.* **2013**, *47*, 957−995.

(4) Pruden, A. Balancing water sustainability and public health goals in the face of growing concerns about antibiotic resistance. *Environ. Sci. Technol.* **2014**, *48*, 5−14.

(5) Nakada, N.; Shinohara, H.; Murata, A.; Kiri, K.; Managaki, S.; Sato, N.; Takada, H. Removal of selected pharmaceuticals and personal care products (PPCPs) and endocrine-disrupting chemicals (EDCs) during sand filtration and ozonation at a municipal sewage treatment plant. *Water Res.* **2007**, *41*, 4373−4382.

(6) Gerrity, D.; Gamage, S.; Holady, J. C.; Mawhinney, D. B.; Quinones, O.; Trenholm, R. A.; Snyder, S. A. Pilot-scale evaluation of ozone and biological activated carbon for trace organic contaminants mitigation and disinfection. *Water Res.* **2011**, *45*, 2155−2165.

(7) Grover, D. P.; Zhou, J. L.; Frickers, P. E.; Readman, J. W. Improved removal of estrogenic and pharmaceutical compounds in sewage effluent by full scale granular activated carbon: impact on receiving river water. *J. Hazard. Mater.* **2011**, *185*, 1005−1011.

(8) Zimmermann, S. G.; Wittenwiler, M.; Hollender, J.; Krauss, M.; Ort, C.; Siegrist, H.; von Gunten, U. Kinetic assessment and modeling of an ozonation step for full-scale municipal wastewater treatment: micropollutant oxidation, by-product formation and disinfection. *Water Res.* **2011**, *45*, 605−617.
(9) Reungoat, J.; Escher, B. I.; Macova, M.; Argaud, F. X.; Gernjak, W.; Keller, J. Ozonation and biological activated carbon filtration of wastewater treatment plant effluents. *Water Res.* **2012**, *46*, 863−872.
(10) Margot, J.; Kienle, C.; Magnet, A.; Mirco, W.; Rossi, L.; de Alencastro, L. F.; Abegglen, C.; Thonney, D.; Chèvre, N.; Schärer, M.; Barry, D. A. Treatment of micropollutants in municipal wastewater: ozone or powdered activated carbon? *Sci. Total Environ.* **2013**, *461*−*462*, 480−498.
(11) Escher, B. I.; Lawrence, M.; Macova, M.; Mueller, J. F.; Poussade, Y.; Robillot, C.; Roux, A.; Gernjak, W. Evaluation of contaminant removal of reverse osmosis and advanced oxidation in full-scale operation by combining passive sampling with chemical analysis and bioanalytical tools. *Environ. Sci. Technol.* **2011**, *45*, 5387−5394.
(12) Yang, X.; Flowers, R. C.; Weinberg, H. S.; Singer, P. C. Occurrence and removal of pharmaceuticals and personal care products (PPCPs) in an advanced wastewater reclamation plant. *Water Res.* **2011**, *45*, 5218−5228.
(13) Gerrity, D.; Pecson, B.; Trussell, R. S.; Trussell, R. R. Potable reuse treatment trains throughout the world. *J. Water Supply Res. Technol.—AQUA* **2013**, *62*, 321−338.
(14) von Sonntag, C.; von Gunten, U. *Chemistry of Ozone in Water and Wastewater Treatment: From Basic Principles to Applications*; IWA Publishing: London, 2012.
(15) Lee, Y.; Gerrity, D.; Lee, M.; Bogeat, A. E.; Salhi, E.; Gamage, S.; Trenholm, R. A.; Wert, E. C.; Snyder, S. A.; von Gunten, U. Prediction of micropollutant elimination during ozonation of municipal wastewater effluents: Use of kinetic and water specific information. *Environ. Sci. Technol.* **2013**, *47*, 5872−5881.
(16) Escher, B. I.; Bramaz, N.; Ort, C. Monitoring the treatment efficiency of a full scale ozonation on a sewage treatment plant with a mode-of-action based test battery. *J. Environ. Monit.* **2009**, *11*, 1836−1846.
(17) Stalter, D.; Magdeburg, A.; Oehlmann, J. Comparative toxicity assessment of ozone and activated carbon treated sewage effluents using an in vivo test battery. *Water Res.* **2010**, *44*, 2610−2620.
(18) Snyder, S. A.; von Gunten, U.; Amy, G.; Debroux, J. *Use of Ozone in Water Reclamation for Contaminant Oxidation: Final Project Report and User Guidance*; WaterReuse Research Foundation: Alexandria, VA, 2012.
(19) Magdeburg, A.; Stalter, D.; Schliusener, M.; Ternes, T.; Oehlmann, J. Evaluating the efficiency of advanced wastewater treatment: Target analysis of organic contaminants and (geno-)toxicity assessment tell a different story. *Water Res.* **2014**, *50*, 35−47.
(20) Lee, Y.; Zimmermann, S. G.; Kieu, A. T.; von Gunten, U. Ferrate (Fe(VI)) application for municipal wastewater treatment: A novel process for simultaneous micropollutant oxidation and phosphate removal. *Environ. Sci. Technol.* **2009**, *43*, 3831−3838.
(21) Sharma, V. K. Oxidation of inorganic compounds by ferrate(VI) and ferrate(V): One-electron and two-electron transfer steps. *Environ. Sci. Technol.* **2010**, *44*, 5148−5152.
(22) Rush, J. D.; Cyr, J. E.; Zhao, Z.; Bielski, B. H. J. The oxidation of phenol by ferrate(VI) and ferrate(V). A pulse radiolysis and stopped-flow study. *Free Radical Res.* **1996**, *22*, 349−360.
(23) Huang, H.; Sommerfeld, D.; Dunn, B. C.; Eyring, E. M.; Lloyd, C. R. Ferrate(VI) oxidation of aqueous phenol: Kinetics and mechanism. *J. Phys. Chem. A* **2001**, *105*, 3536−3541.
(24) Huang, H.; Sommerfeld, D.; Dunn, B. C.; Lloyd, C. R.; Eyring, E. M. Ferrate(VI) oxidation of aniline. *J. Chem. Soc., Dalton Trans.* **2001**, 1301−1305.
(25) Sharma, V. K.; Rivera, W.; Smith, J. O.; O'Brien, B. Ferrate(VI) oxidation of aqueous cyanide. *Environ. Sci. Technol.* **1998**, *32*, 2608−2613.
(26) Johnson, M. D.; Hornstein, B. J. The kinetics and mechanism of the ferrate(VI) oxidation of hydroxylamines. *Inorg. Chem.* **2003**, *42*, 6923−6928.
(27) Johnson, M. D.; Bernard, J. Kinetics and mechanism of the ferrate oxidation of sulfite and selenite in aqueous media. *Inorg. Chem.* **1992**, *31*, 5140−5142.
(28) Lee, Y.; Yoon, J.; Von Gunten, U. Kinetics of the oxidation of phenols and phenolic endocrine disruptors during water treatment with ferrate (Fe(VI)). *Environ. Sci. Technol.* **2005**, *39*, 8978−8984.
(29) Sharma, V. K.; Mishra, S. K.; Nesnas, N. Oxidation of sulfonamide antimicrobials by ferrate(VI) [$Fe^{VI}O_4^{2-}$]. *Environ. Sci. Technol.* **2006**, *40*, 7222−7227.
(30) Li, C.; Li, X. Z.; Graham, N.; Gao, N. Y. The aqueous degradation of bisphenol A and steroid estrogens by ferrate. *Water Res.* **2008**, *42*, 109−120.
(31) Hu, L.; Martin, H. M.; Arce-Bulted, O.; Sugihara, M. N.; Keating, K. A.; Strathmann, T. J. Oxidation of carbamazepine by Mn(VII) and Fe(VI): Reaction kinetics and mechanism. *Environ. Sci. Technol.* **2009**, *43*, 509−515.
(32) Anquandah, G. A. K.; Sharma, V. K.; Knight, D. A.; Batchu, S. R.; Gardinali, P. R. Oxidation of trimethoprim by ferrate(VI): Kinetics, products, and antibacterial activity. *Environ. Sci. Technol.* **2011**, *45*, 10575−10581.
(33) Yang, B.; Ying, G. G.; Zhao, J. L.; Zhang, L. J.; Fang, Y. X.; Nghiem, L. D. Oxidation of triclosan by ferrate: Reaction kinetics, products identification and toxicity evaluation. *J. Hazard. Mater.* **2011**, *186*, 227−235.
(34) Yang, B.; Ying, G. G.; Zhang, L. J.; Zhou, L. J.; Liu, S.; Fang, Y. X. Kinetic modeling and reaction mechanism of ferrate(VI) oxidation of benzotriazoles. *Water Res.* **2011**, *45*, 2261−2269.
(35) Zimmermann, S. G.; Schmukat, A.; Schulz, M.; Benner, J.; von Gunten, U.; Ternes, T. A. Kinetic and mechanistic investigations of the oxidation of tramadol by ferrate and ozone. *Environ. Sci. Technol.* **2012**, *46*, 876−884.
(36) Anquandah, G. A. K.; Sharma, V. K.; Panditi, V. R.; Gardinali, P. R.; Kim, H.; Oturan, M. A. Ferrate(VI) oxidation of propranolol: kinetics and products. *Chemosphere* **2013**, *91*, 105−109.
(37) European Centre for Disease Prevention and Control. *Surveillance of Antimicrobial Consumption in Europe 2011*; ECDC: Stockholm; 2014. Available at http://www.ecdc.europa.eu/en/publications/Publications/antimicrobial-consumption-europe-surveillance-2011.pdf.
(38) CDDEP. *Antibiotic use overview*. Available at http://www.cddep.org/resistancemap/use#.U4qGtvl_scY.
(39) Walsh, C. *Antibiotics: Actions, Origins, Resistance*; ASM Press: Washington, DC, 2003.
(40) Hirsch, R.; Ternes, T.; Haberer, K.; Kratz, K.-L. Occurrence of antibiotics in the aquatic environment. *Sci. Total Environ.* **1999**, *225*, 109−118.
(41) Cha, J. M.; Yang, S.; Carlson, K. H. Trace determination of β-lactam antibiotics in surface water and urban wastewater using liquid chromatography combined with electrospray tandem mass spectrometry. *J. Chromatogr. A* **2006**, *1115*, 46−57.
(42) Zuccato, E.; Castiglioni, S.; Fanelli, R. Identification of the pharmaceuticals for human use contaminating the Italian aquatic environment. *J. Hazard. Mater.* **2005**, *122*, 205−209.
(43) Andreozzi, R.; Caprio, V.; Ciniglia, C.; de Champdoré, M.; lo Giudice, R.; Marotta, R.; Zuccato, E. Antibiotics in the environment: occurrence in Italian STPs, fate, and preliminary assessment on algal toxicity of amoxicillin. *Environ. Sci. Technol.* **2004**, *38*, 6832−6838.
(44) Watkinson, A. J.; Murby, E. J.; Costanzo, S. D. Removal of antibiotics in conventional and advanced wastewater treatment: Implications for environmental discharge and wastewater recycling. *Water Res.* **2007**, *41*, 4164−4176.
(45) Gulkowska, A.; Leung, H. W.; So, M. K.; Taniyasu, S.; Yamashita, N.; Yeunq, L. W. Y.; Richardson, B. J.; Lei, A. P.; Giesy, J. P.; Lam, P. K. S. Removal of antibiotics from wastewater by sewage treatment facilities in Hong Kong and Shenzhen, China. *Water Res.* **2008**, *42*, 395−403.

(46) Benitez, F. J.; Acero, J. L.; Real, F. J.; Roldan, G.; Casas, F. Bromination of selected pharmaceuticals in water matrices. *Chemosphere* **2011**, *85*, 1430−1437.
(47) Acero, J. L.; Benitez, F. J.; Real, F. J.; Roldan, G. Kinetics of aqueous chlorination of some pharmaceuticals and their elimination from water matrices. *Water Res.* **2010**, *44*, 4158−4170.
(48) Navalon, S.; Alvaro, M.; Garcia, H. Reaction of chlorine dioxide with emergent water pollutants: Product study of the reaction of three β-lactam antibiotics with ClO_2. *Water Res.* **2008**, *42*, 1935−1942.
(49) Sharma, V. K.; Liu, F.; Tolan, S.; Shon, M.; Kim, H.; Oturan, M. A. Oxidation of β-lactam antibiotics by ferrate(VI). *Chem. Eng. J.* **2013**, *221*, 446−451.
(50) Dodd, M. C.; Buffle, M. O.; 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*, 1969−1977.
(51) Dodd, M. C.; Kohler, H. E.; 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*, 2498−2504.
(52) Dodd, M. C.; Rentsch, D.; Singer, H. P.; Kohler, H. E.; von Gunten, U. Transformation of β-lactam antibacterial agents during aqueous ozonation: Reaction pathways and quantitative bioassay of biologically-active oxidation products. *Environ. Sci. Technol.* **2010**, *44*, 5940−5948.
(53) Dail, M. K.; Mezyk, S. P. Hydroxyl-radical-induced degradative oxidation of β-lactam antibiotics in water: absolute rate constant measurements. *J. Phys. Chem. A* **2010**, *114*, 8391−8395.
(54) Rickman, K. A.; Mezyk, S. P. Kinetics and mechanisms of sulfate radical oxidation of β-lactam antibiotics in water. *Chemosphere* **2010**, *81*, 359−365.
(55) Lee, Y.; Yoon, J.; von Gunten, U. Spectrophotometric determination of ferrate (Fe(Vl)) in water by ABTS. *Water Res.* **2005**, *39*, 1946−1953.
(56) Flyunt, R.; Leitzke, A.; Mark, G.; Mvula, E.; Reisz, E.; Schick, R.; von Sonntag, C. Determination of $^{\bullet}OH$, $O_2^{\bullet-}$, and hydroperoxide yields in ozone reactions in aqueous solution. *J. Phys. Chem. B* **2003**, *107*, 7242−7253.
(57) American Public Health Association (APHA). *Standard Methods for the Examination of Water and Wastewater*, 20th ed.; APAH: Washington, DC, 1998.
(58) Sharma, V. K.; Burnett, C. R.; Millero, F. J. Dissociation constants of the monoprotic ferrate(VI) ion in NaCl media. *Phys. Chem. Chem. Phys.* **2001**, *3*, 2059−2062.
(59) Lee, Y.; von Gunten, U. Oxidative transformation of micropollutants during municipal wastewater treatment: Comparison of kinetic aspects of selective (chlorine, chlorine dioxide, ferrate^{VI}, and ozone) and non-selective oxidants (hydroxyl radical). *Water Res.* **2010**, *44*, 555−566.
(60) Thompson, J. E. *A Practical Guide to Contemporay Pharmacy Practice*; Lippincott Williams & Wilkins: Philadelphia, 1998.
(61) Cabot, J. M.; Fuguet, E.; Rafols, C.; Roses, M. Determination of acidity constants by the capillary electrophoresis internal standard method. IV. Polyprotic compounds. *J. Chromatogr. A* **2013**, *1279*, 108−116.
(62) Lee, Y.; von Gunten, U. Quantitative structure-activity relationships (QSARs) for the transformation of organic micropollutants during oxidative water treatment. *Water Res.* **2012**, *46*, 6177−6195.
(63) Lee, Y.; Kissner, R.; von Gunten, U. Reaction of ferrate(VI) with ABTS and self-decay of ferrate(VI): Kinetics and mechanisms. *Environ. Sci. Technol.* **2014**, *48*, 5154−5162.
(64) Carr, J. D.; Kelter, P. B.; Ericson, A. T. Ferrate(VI) oidation of nitrilotriacetic acid. *Environ. Sci. Technol.* **1981**, *15*, 184−187.
(65) Hornstein, B. J. Reaction mechanisms of hypervalent iron: The oxidation of amines and hydroxylamines by potassium ferrate, K_2FeO_4; Ph.D. Thesis, New Mexico State University: Las Cruces, NM, 1999.
(66) Noorhasan, N.; Patel, B.; Sharma, V. K. Ferrate(VI) oxidation of glycine and glycylglycine: Kinetics and products. *Water Res.* **2010**, *44*, 927−935.
(67) Coghill, R. D.; Stodola, F. H.; Wachtel, J. L. In *The Chemistry of Penicillin*; Clarke, H. T., Johnson, J. R., Robinson, R., Eds.; Princeton University Press: Princeton, 1949; pp 680−687.
(68) Chow, A. W.; Hoover, J. R. E.; Hall, N. M. Penicillin sulfoxides and sulfones. *J. Org. Chem.* **1962**, *27*, 1381−1383.