Oxidation towards enrofloxacin degradation over nanoscale zero-valent copper: Mechanism and products

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Abstract

Enrofloxacin (ENR) is a widely used veterinary fluoroquinolone antibiotic and is frequently detected in water environments. The degradation of ENR was examined utilizing molecular oxygen mediation using nanometer zero-valent copper (nZVC) as the catalyst in this work. The dosage of nZVC, initial pH, and reaction temperature were investigated as contributing factors to ENR degradation. The reactive oxygen species (ROS) that participated in the reaction were identified, their generation mechanisms were elucidated, and the effects on ENR degradation were assessed. More emphasis was given to exploring ENR degradation and transformation pathways via analyses of HPLC-TOF-MS. Data showed that at 35 °C, with an initial pH of 3 and exposed to air, an nZVC dose of 0.5 g·L⁻¹ degraded ENR by 99.51% dramatically. HO• radicals were identified as the dominant ROS, and conversions among Cu⁰, Cu⁺, and Cu²⁺ played crucial roles in the generation of ROS. The destruction mechanism of ENR was speculated based on analyses of HPLC-TOF-MS results as the transformation of the piperazine ring into an oxidized state with a -COOH substitution with HO•, which caused ENR to be mineralized and converted into CO₂, H₂O, and NO₃⁻. This research proposes a capable and practical method for removing ENR from water.

1. Introduction

Enrofloxacin (ENR) is the first animal-specific fluoroquinolone antibiotic widely applied in livestock and poultry breeding and aquaculture for the prevention and treatment of bacterial diseases and mycoplasma infections in animals. After ingesting in animals, a large proportion of ENR will enter the environment, especially in water, in the form of prodrugs and metabolites, which could make microorganisms resistant to drugs and affect plant and human health (Du and Liu 2012; Qiao et al. 2018; Wang et al. 2021). Since ENR was lipophilic, it is not easily degraded in the water environment. Nowadays, ENR is identified regularly in surface waters, drinking water, wastewater, and other aquatic habitats (Li et al. 2020; Zhu et al. 2021; Sodhi and Singh 2021). The transformation and removal of ENR in water have attracted intense attentions of many scholars.

Antibiotic elimination strategies include the Fenton process (de Melo Costa-Serge et al. 2021), ferrate(VI) (Yang et al. 2016; Suyamud et al. 2021), activated persulfate oxidation (Zhou et al. 2018b; Gao et al. 2022), electrochemical oxidation (Wang et al. 2017; Xu et al. 2022), etc. Molecular oxygen (O₂) is a green and environment-friendly oxidant with several applications in pollutant degradation (Khachatryan et al. 2011; Zhang et al. 2021a). However, the oxidant capacity of molecular oxygen is not satisfactory, and Molecular oxygen is unable to degrade some refractory organics directly (Li and Li 2021). Therefore, converting molecular oxygen into reactive ROS with substantial oxidant capability is essential to effectively and efficiently break down contaminants. For ROSs generation, many methods have been proposed, including the electrochemical process (Ouyang et al. 2019), photocatalysis (Di et al. 2016; Liu et al. 2021a), and metal catalysis (Zhang et al. 2020a; Yang et al. 2022b; Yu et al. 2022). Among these methods, metal catalysis is considered an economical and convenient way were owing to the high efficiency, easy availability, and simple modification of metallic materials.
Nowadays, zero-valent metals are commonly used to activate molecular oxygen, such as zero-valent iron (ZVI) (Gao et al. 2020; Liu et al. 2022), zero-valent aluminum (ZVA) (Zhang et al. 2020b; Ileri and Dogu 2022), and zero-valent copper (ZVC) (Long et al. 2020; Zhang et al. 2021a). With $E_0$ of +0.337 V, ZVC exhibits superior catalytic performance compared to ZVI of -0.44 V and ZVA of -1.667 V (Zhang et al. 2017). Previous research has demonstrated that the large bonds in the aromatic rings of target organic pollutants are able to form stronger conjugations with the electronic orbitals of ZVC, making electron exchange more straightforward than it would be with ZVI and so contributing to copper's high degradation efficiency (de Souza et al. 2021; Weidlich 2021). Moreover, ZVC permits a broader pH reaction range than ZVI, which is advantageous for practical applications. Diethyl phthalate breakdown in water-soluble ZVC under aerobic atmospheric conditions was studied by Wen et al. (Wen et al. 2014), and the mechanism analysis revealed that a significant amount of $\text{Cu}^{+}/\text{Cu}^{2+}$ and $\text{H}_2\text{O}_2$ were produced in acidic solutions. Smaller particles, on the other hand, may offer more active areas on the surface and enhance ROS production (Yu et al. 2020). Dong and colleagues (Dong et al. 2014) reported the elimination of azo pollutants by nanoscale zero-valent copper (nZVC) at neutral pH. The contamination-dependent performance of nZVC was connected to the production of Cu(I) and $^\cdot\text{OH}$ via nZVC-induced activation of $\text{O}_2$. However, there are very few studies have been conducted on the degradation of ENR in the nZVC/$\text{O}_2$ system, and the principal reactive species, degradation mechanisms, and pathways of ENR in such a system are still unclear.

In this study, different aspects of nZVC/$\text{O}_2$ system on ENR degradation have been evaluated, including effects of reaction conditions, ROS produced in the system, and generation mechanism of ROS. More emphasis was given to exploring ENR destruction and transformation pathways via analyses of ENR degradation intermediates using HPLC-TOF-MS. The acquired results would enhance our understanding of the decomposition of ENR with molecular oxygen activated by nZVC and give a novel strategy for removing ENR from water.

2. Material And Methods

2.1. Chemicals

All of the reagents listed here are of analytical grade, including acetonitrile ($\text{CH}_3\text{CN}$), methanol ($\text{CH}_3\text{OH}$), and formic acid ($\text{HCOOH}$), provided the materials by Sigma-Aldrich Chemical Co. Ltd., (Shanghai, China). nZVC ($\geq 98\%$ purity, 10–30 nm) was obtained from Macklin Biochemical Co. Ltd., (Shanghai, China). The acquisition of ENR was from Dr. Ehrenstorfer GmbH (Germany). Other chemical reagents involving tert-butanol (TBA) and p-benzoquinone (p-BQ) were obtained from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). All experiments were performed with ultrapure water generated from the Milli-Q system.

2.2. Batch experiments
The oxygen activation by nZVC was evaluated via the ENR degradation. All experiments were conducted under an ambient atmosphere. In the degradation experiment, an appropriate amount of nZVC was combined with 100 mL of ENR (10 mg·L\(^{-1}\)) in a conical glass flask. Using HCl or NaOH, the initial pH was adjusted to the desired value. At a constant temperature, all the glass flasks were loaded in an automated shaker. The solution was held in an open state during the procedure (60 min) to ensure enough dissolved oxygen. Samples of 1 mL were taken out and quenched by excess sodium thiosulfate at a certain time interval. The samples were extracted with 30 mL of ethyl acetate to facilitate identification of the transformation products. The extracted samples were then concentrated and collected in 1 mL of methanol before being analyzed after filtering through a 0.22 µm filter. To determine the effect of coexisting ions and natural organic matters (NOM) on ENR degradation, 1 mmol·L\(^{-1}\) Cl\(^-\), NO\(_3\)^- , SO\(_4^{2-}\) (each from NaCl, NaNO\(_3\), Na\(_2\)SO\(_4\)) or 10 mg·L\(^{-1}\) humic acid was added to the solutions at pH = 3. Other conditions were the same as those of the batch experiments with nZVC. The experiments were repeated three times for accuracy.

### 2.3. ROS determination

Quenching experiments were performed to validate the production of ROSs. TBA and p-BQ were used to quench hydroxyl radicals (HO\(^\cdot\)) and superoxide anion radicals (O\(_2^\cdot\)), respectively. One scavenger was added to the solution at the start of the quenching tests, but all other protocols were kept the same. The active radicals were identified using electron paramagnetic resonance (EPR, Bruker ESP-300E, Germany) spectroscopy, and 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) was used to spin trap HO\(^\cdot\) and O\(_2^\cdot\) radicals in order to form corresponding adducts. The EPR measurements were performed using 10 mW of microwave power, 100 G of scan range, and 1 G of field modulation.

### 2.4. Analytical methods

An Agilent 2000 HPLC system determined the concentration of ENR at 254 nm detection wavelength, XDB-C18 reversed-phase column (4.5×150 mm, 5µm, Waters, USA). At a flow rate of 0.75 mL·min\(^{-1}\), the mobile phase was an 85:15 combination of triethylamine solution (containing 0.34% phosphoric acid) and acetonitrile. The ENR degradation process is represented by the pseudo-first-order kinetic model in Eq. (1).

\[
\ln \frac{C_t}{C_0} = -kt
\]

Where \(C_t\) is the concentration of ENR in the solution at time \(t\), \(C_0\) is the initial concentration of ENR in the solution, \(t\) is the reaction time in minutes, and \(k\) is the pseudo-first-order rate constant (min\(^{-1}\)).

The transformation products of ENR degradation and their distributions were determined by HPLC equipped with a time-of-flight mass spectrometer (HPLC-TOF-MS). This instrument was composed of an Agilent 2000series HPLC and a TOF System (AB SCIEX Triple 5600). The transformation products were
separated on an ACQUITY UPLC BEH C18 column (100 mm×2.1mm, particle size 1.7 µm, Waters, USA) in an electrospray positive ion mode (ESI+). The temperature of the chromatographic column was set at 30°C, the mobile phase was 0.1% formic acid aqueous solution and methanol, the flow rate was 0.200 mL·min⁻¹, and the injection volume was 20µL. The Source was kept at a temperature of 30°C. A gradient elution was programmed as follows: 5% methanol for the first 2 minutes; 2–25 min, 5% methanol; 25–35 min, 95% methanol; 35-35.01 min, 5% methanol; 35.01-40 min, 5% methanol. Full mass spectra were acquired from m/z 0 to 1000.

Besides, ICP-OES (PerkinElmer 8300, USA) was adopted to determine Cu concentration in the solution, and X-ray photoelectron spectroscopy (XPS, K-Alpha, Thermo, America) analyses were performed to accurately determine the composition and the valence state of Cu after the reaction.

2.5 Characterization

The morphological information of the nZVC particles was characterized using a SU8000 field emission scanning electron microscope (FESEM; TESCAN MIRA LMS, Czech Republic) equipped with an energy dispersive spectrometer (EDS, Oxford Xplore 30, UK). The composition of the nZVC powders was identified by X-ray diffraction (XRD) analysis (Rigaku SmartLab SE, Japan) using Cu-Kα as the source of radiation and at a scanning range of 2θ from 10 to 80°.

3. Result And Discussion

3.1 Characterization of the nZVC

FESEM and XRD were used to characterize the morphological properties and phase structure of nZVC powders. As shown in Fig. 1(a-c), FESEM images revealed that nZVC powders consist of many irregular clumps, which are composed of spherical particles of different sizes. As seen in Fig. 1e, nZVC particles have a diameter of approximately 30 nm. Furthermore, as shown in Fig. 2a, three diffraction peaks at 43.40°, 50.54°, and 74.22° can be visible in the XRD pattern of nZVC, which correspond to several major Cu⁰ diffraction peaks (JCPDS-PDF#04-0836), indicating that nZVC powders are made chiefly of Cu⁰ (Zhou et al. 2018a). Also, minor peaks at 36.48°, 42.40°, and 61.42°, as well as those at 37.50°, 46.00°, and 48.48° corresponded well to the standard pattern of Cu₂O (JCPDS-PDF#05-0667) and Cu₂S ((JCPDS-PDF#33–0490), respectively. Furthermore, EDS analysis shows that the nZVC contains a small amount of O, and S elements(Fig. 1d), which may be some Cu₂O coating attached to the surface of nZVC and a few Cu₂S impurities in nZVC powders(Sousa et al. 2019). However, the XRD pattern of nZVC did not display the expected CuO diffraction peaks. Thus, Cu⁰ made up the bulk of nZVC powders (Zhang et al. 2021b).

3.2. Performance and mechanism of nZVC/O₂ system

The performances of different systems were compared for ENR degradation, as shown in Fig. 2b. The ENR degradation was found to be less than 10% within 60 minutes in the absence of nZVC, indicating
that dissolved O\textsubscript{2} alone could not effectively degrade ENR. With nZVC addition, the elimination efficiencies of ENR significantly increased to 86.42% within 60 min. Meanwhile, a contrast experiment of nZVC under anaerobic conditions was carried out, 20 minutes of nitrogen aeration were performed prior to the addition of nZVC to remove dissolved oxygen from the solution, and the reactor was sealed during the process. The change curve of the anaerobic reaction system indicated that the nZVC had negligible adsorption capacity to ENR. Therefore, the degradation of ENR in nZVC/O\textsubscript{2} system might be due to the reaction between nZVC and O\textsubscript{2} to produce a series of ROS, and the catalyst was an essential factor for efficient degradation.

In order to further explore the ROS that plays a leading role in the degradation process, the concentrations of Cu\textsuperscript{+} and H\textsubscript{2}O\textsubscript{2} produced at different reaction times in nZVC/Air system were determined by spectrophotometry. As shown in Fig. 2c, H\textsubscript{2}O\textsubscript{2} is indeed produced in the reaction system, and its concentration varies between 0.05-0.65mM. The contents of Cu\textsuperscript{+} and H\textsubscript{2}O\textsubscript{2} increased rapidly within 0–10 minutes and reached the maximum value about 10 minutes later, and then gradually decreased.

In general, hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}), hydroxyl (HO\textsuperscript{•}), and superoxide (O\textsubscript{2} \textsuperscript{•−}) are the possible ROS in zero-valent metal/O\textsubscript{2} system (Liu et al. 2021b; Li et al. 2021), and their generation route has been reported as follows: to begin, nZVC leaching in acidic conditions generates Cu\textsuperscript{+} and Cu\textsuperscript{2+} as Eqs. (2)-(3); simultaneously, O\textsubscript{2} is spontaneously reduced to H\textsubscript{2}O\textsubscript{2} (Eq. (4)); then, some Fenton-like reactions occurred, such as the interaction between Cu\textsuperscript{+} and H\textsubscript{2}O\textsubscript{2} (Eq. (5)) that resulted in the production of HO\textsuperscript{•}, or the reaction between Cu\textsuperscript{2+} and H\textsubscript{2}O\textsubscript{2} (Eq. (6)), which gave O\textsubscript{2} \textsuperscript{•−}. Meanwhile, the latter would be further transformed into the hydroperoxyl radical (H\textsubscript{2}O\textsubscript{2} \textsuperscript{•}) in acidic media (Eq. (7)).

\begin{align*}
\text{Cu}^0 & \rightleftharpoons \text{Cu}^+ + e^- E^0 = \text{-0.528 V} \\
\text{Cu}^0 & \rightleftharpoons \text{Cu}^{2+} + 2e^- E^0 = \text{-0.334 V} \\
\text{O}_2 + 2\text{H}^+ + 2e^- & \rightleftharpoons \text{H}_2\text{O}_2 E^0 = \text{+0.695 V} \\
\text{Cu}^+ + \text{H}_2\text{O}_2 & \rightleftharpoons \text{Cu}^{2+} + \text{HO}^\bullet + \text{HO}^- \\
\text{Cu}^{2+} + \text{H}_2\text{O}_2 & \rightleftharpoons \text{Cu}^+ + \text{O}_2^{•−} + \text{H}^+ 
\end{align*}
Although all of these radical species can then take part in ENR destruction, HO⁻ radicals had the largest redox potential and were the most reactive to organic molecules (Zhang et al. 2021a), therefore possibly played a more dominant role in ENR degradation. To validate this hypothesis, quenching experiments were done with two well-known scavengers present, TBA for HO⁻ and p-BQ for O₂•-. As can be seen in Fig. 2e, when TBA was present, the efficiency with which ENR was degraded dropped to approximately 15%, whereas the inhibitory impact of p-BQ was diminished, and the rate at which ENR was removed was around 58%. Subsequently, EPR spectra with DMPO spin trapping adducts was adopted to confirm the existence of these ROS intuitively. A four-line spectrum with relative intensities of 1:2:2:1 was found from nZVC aqueous dispersions, indicating the production of HO⁻ (Fig. 2f). Besides, six characteristic peaks of DMPO-O₂•⁻ adducts were also identified (Fig. 2g).

Additionally, under aerobic air circumstances, the oxidation capacity of the nZVC acidic system was significantly impacted by the presence of Cu⁺ and Cu²⁺, as shown by Eqs. (2)-(7). Binding energies of the copper after using (Fig. 2d) at roughly 932.2 eV and 935.3 eV were assigned to the Cu 2p₃/₂ orbital in Cu₂O and CuO, respectively, revealing the co-existence of Cu⁺ and Cu²⁺ in the system.

3.3. Effect of different conditions on ENR degradation

3.3.1. Effect of initial pH

As known, pH is a crucial aspect concerning the elimination performance of the oxidation unit. Herein, the impact of initial pH on ENR degradation was examined by introducing varied quantities of HCl or NaOH to the reaction system at 15°C. As shown in Fig. 3a, the nZVC/O₂ degradation system was active between the pH ranges of 3.08 and 8.96, and lower pH values aided the degrading process, while almost no degradation was performed at a pH of 10.98. Besides, Cu concentration in the solution after the reaction was measured using ICP-OES, and the results are presented in Fig. 3b. With the increasing pH value, Cu concentration significantly decreased, and trace Cu was detected under alkaline conditions. Moreover, it was discovered that the final pH was higher following the reaction, regardless of the starting pH, which was attributed to the consumption of H⁺ ions.

Since H⁺ could dissolve the oxide layer on the surface of the zero-valent metal (Deng et al. 2019; Sun et al. 2021), it could be envisaged that hydronium ion dissolved the oxide layer on the surface of Cu⁰ and promoted degradation of ENR. Furthermore, high concentrations of H⁺ may enhance the formation of H₂O₂, which in turn promotes the degradation of organic matter (Zhou et al. 2016; Guo et al. 2021). Under strong alkaline conditions, OH⁻ would react with Cu⁺ to generate cuprous hydroxide (CuOH), which could spontaneously decompose into Cu₂O and H₂O, significantly inhibiting the ENR degradation (Zhang et al. 2021a, b). In summary, acidic condition facilitated the ENR removal. Wen et al. (Wen et al. 2014) observed comparable results when utilizing ZVC to degrade diethyl phthalate, while Sousa et al. (Sousa et al. 2019) discovered a drop in the proportion of ciprofloxacin degradation when applying nZVC. Additionally, the
change in pH of the solution was observed, and an increase was detected following the reaction. This suggests that H⁺ was used in the nZVC/O₂ system, as the rise in pH indicates. Although the dissolved Cu concentrations in acidic solutions were higher than those allowed in the Chinese discharge standard for wastewater (2.0 mg·L⁻¹), which may raise questions about the nZVC application Cu⁺/Cu²⁺ might be readily eliminated by precipitation techniques, such as combining with a solution containing sulfide or phosphorus and adjusting the pH value (Hollanda et al. 2019). The previous methodology was used for this investigation, and over 99.98% of Cu in the solution was precipitated. Subsequent experiments were conducted with initial pH of 3. Over 99.98% of the Cu in the solution was precipitated using the former method used in the present investigation. In subsequent trials, an initial pH of 3 was used.

### 3.3.2. Effect of nZVC dosage

The impact of nZVC dose (0.10–0.90 g·L⁻¹) on ENR degradation at an initial pH of 3 is shown in Fig. 3c. Within 60 minutes, the elimination efficiency of ENR improved from 57.23–99.70% as the dosage of nZVC went from 0.10 g·L⁻¹ to 0.90 g·L⁻¹. Furthermore, the pseudo-first-order kinetic was employed to calculate the ENR degradation rate constant, k. As shown in Fig. 3d, the k value was 0.01, 0.03, 0.06, 0.15, and 0.22 min⁻¹ with nZVC dosage of 0.10, 0.30, 0.50, 0.70, and 0.90 g·L⁻¹, respectively. According to Yang et al. (Yang et al. 2022b) and Zhang et al. (Zhang et al. 2021b), the increasing degradation with ZVC concentration was likely due to the enhanced production of Cu⁺/Cu²⁺, which could promote the reaction of molecular oxygen with water to form ROS.

### 3.3.3. Effect of temperature

The catalysis of molecular oxygen and the generation of ROS are processes that are greatly influenced by temperature. The influence of temperature on ENR degradation in the range of 15–45°C was investigated, and Fig. 4a-b reveals that the temperature of the reaction played a significant role in ENR degradation. At higher reaction temperatures, higher degradation efficiency occurred. The ENR degradation at 15, 25, 35, and 45°C within 30 min was approximately 83.24%, 88.33%, 99.51%, and 99.85%, respectively. Moreover, with the increasing reaction temperature, the degradation of ENR significantly accelerated. ENR degradation in each temperature was accompanied by pseudo-first-order kinetic, and the k₀obs increased from 0.06 to 0.14 min⁻¹ with enhancing reaction temperature. These results may be explained by the higher dissolution of Cu and generation of ROS at higher reaction temperatures (Deng et al. 2019), which could further promote the degradation of ENR. Satisfactory ENR degradation could be received at 35°C.

### 3.3.4. Effect of co-exiting anions and NOM

Commonly, certain anions and NOM coexisted in natural water, which may affect the system's oxidation reaction performance. In order to validate the influence of typical anions (Cl⁻, NO₃⁻, SO₄²⁻) and NOM (humic acid) on ENR elimination by the nZVC/O₂ system, tests were conducted. According to the results presented in Fig. 4c, the presence of Cl⁻ had a positive impact on the ENR degradation, but NO₃⁻ and SO₄²⁻ exhibited little influence, implying that they do not interfere in the system. These findings align with the phenomena described by Wen et al. (Wen et al. 2014) and Sousa et al. (Sousa et al. 2019). The Cl⁻
accelerated the degradation reaction, as it could stabilize Cu\(^+\) and prevent Cu\(^{2+}\) oxidation (Zhang et al. 2020c). Cu\(^+\) would react with H\(_2\)O\(_2\) and O\(_2\) already there to make HO\(^-\) and O\(_2\)^•-. Significantly detrimental effects were detected for humic acid, mainly owing to rivalry between humic acid and ENR for HO\(^-\) radicals (Zhuan and Wang 2020).

3.4. Identified of ENR degradation products

The complexity of partially oxidized manufacturing products during ENR oxidation was essential to considering the reaction mechanism. The use of HPLC-Q-TOF-MS is possible to track these plausible oxidation products. The reactor did not find ENR after 60 minutes. The potential metabolites of ENR were screened and analyzed with secondary mass spectrometry based on variations in sample primary mass spectra. The probable molecular structure of the substance was estimated following the structure of the parent compound. While, several oxidation transformation products were identified with the help of Analyst TF 1.7.1 software in the nZVC/O\(_2\) system, as shown by peak deconvolution and alignment of the chromatograms obtained from the treated and control samples (Table 1 and Table S1). Fig. S1 describes the hypothesized structures for the fragment ions of the protonated ENR, and Fig. S2 presents a summary of the MS/MS spectra of the primary ENR dissociation products as determined by HPLC-Q-TOF-MS.

The structure of ENR is based on cyclopropyl at the N1 position, fluorine substituent at C6, N-ethyl piperazine at C7, and 4-oxo-quinoline-3-carboxylic acid (Fig. S3). According to previous literature (Sturini et al. 2010; Morales-Gutiérrez et al. 2014; Yang et al. 2016), the quinolone ring and the piperazine ring of the molecule are both potential sites for ENR degradation. The accurate mass data analysis of the MS/MS spectrum of ENR can prove that the carboxyl substituent of ENR at C3 is easily lost, and the loss of CO\(_2\) and H\(_2\)O results in fragmented molecules of \(m/z\) 316 and \(m/z\) 342 (Fig. S2). Additionally, the further loss may also include the cleavage of the piperazinyl moiety, and the loss of the piperazine ring to different degrees forms fragment molecules of \(m/z\) 245 and \(m/z\) 286 substituted by N-vinyl and N-amino vinyl at C7, respectively. It is possible to classify this as secondary fragmentation. At the very end, the cyclopropyl group is lost, splitting from \(m/z\) 245 to \(m/z\) 203. (Fig. S1)

The identification of ENR's degradation products is given a lot of attention. Studies on ENR have indeed been reported in the presence of biochar (Xiao et al. 2020), graphene-WO\(_3\) nanocomposites (Guo et al. 2019), UV (Guo et al. 2017), lead dioxide anode (Wang et al. 2017), ferrate(VI) (Yang et al. 2016), and peroxymonosulfate (Zhou et al. 2018b). The degradation products of ENR were analyzed based on their accurate-mass data of the MS/MS spectra as well as ENR, and all results summarized in Fig. S2.

3.5. Proposed reaction pathway for ENR degradation in nZVC/O\(_2\) system

The major ENR degradation pathway may be mainly piperazine side-chain oxidation (Junza et al. 2016; Zhang et al. 2021a). Therefore, the generated transformation products would be ascribed to the cleavage of piperazine rings. In fact, the sequence of 2H loss as a transition state of the following degradation
product is noteworthy to notice; accurate-mass measurements support this pattern. (P6 in Fig. S1 and Fig. S2). P6 ([M + H]^+ with m/z 358) exhibits subsequent addition of two OH leading to fragment ions with m/z 392 at the presence of nZVC. Immediately afterward, the cleavage of the piperazine ring, two N-C = O species (P5, m/z = 390) would be formed, but two times CO may lose the piperazine ring and generate an amino group (P1, m/z = 334). After that, the loss of CH = N-C=O,N and 2H, and the addition of 0 would be formed and generate an HN-C = O species group (P8, m/z = 291). In the end, the loss of CO would be formed and generate an amine species group (P7, m/z = 263). It should be mentioned that m/z = 263 was the most generally reported intermediate product in ENR oxidative degradation such as electrochemical degradation (Wang et al. 2017), peroxymonosulfate (Zhou et al. 2018b; Nihemaiti et al. 2020), and ferrate (Yang et al. 2016, 2022a). A possible reaction mechanism for ENR degradation has been suggested, based on the chromatogram and mass spectrum obtained in conjunction with the current literature. In Fig. 5, the proposed manufacturing goods were named “P” (where P means proposed). The conversion diagram of several main dissociation products of ENR by HPLC-Q-TOF-MS is described in Fig. S1, and their MS/MS spectra of primary dissociation products of ENR Fig. S2.

Utilizing HPLC-Q-TOF-MS, several transformation products were also detected during ENR degradation. The piperazine ring and carboxylic group of ENR were the main sites in the degradation process. ENR could be mineralized to utilize CO₂, H₂O, and NO₃⁻ through oxidation of the piperazine ring or hydroxylation (Wang et al. 2017). Notably, removing of -COOH on the quinolone ring or the substitution of HO⁻ was also one of the processes of ENR degradation. Generally, the piperazine ring could be hydroxylated to break the N-C bond (Pathway I). This degradation pathway involves the loss of the piperazinyl group. The secondary amine N and tertiary amine N at the piperazine ring were easily attacked by 'OH, which may form two hydroxyls product (P3 m/z = 392). This intermediate formed the P3 by further decarboxylation reaction. Subsequently, the CH₂-NH-C₂H₅ at C7 was cleaved and further oxidized with the loss of H to generate P8 (m/z = 291). P7 (m/z = 263) had the same product, which may prove that the complete loss of the piperazine ring was caused by the loss of the CO fragment in P7 (m/z = 263). Similarly, cleavage of the piperazine ring has been reported by various authors (Jiang et al. 2016; Fang et al. 2021; Shu et al. 2021). Pathway II may be hydrolyzed and form P4 (m/z = 376). In pathway II, a piperazine ring was attacked by HO⁻ and produced the hydroxylated intermediate.

4. Conclusion

In this work, the degradation of ENR, a widely used veterinary fluoroquinolone antibiotic, was investigated by molecular oxygen mediation using nanometer zero-valent copper (nZVC) as the catalyst. Batch experiments showed that the degradation efficiency of ENR was significantly accelerated by increasing nZVC dosage, acidity, and reaction temperature. The degradation efficiency of ENR (10 mg/L) was achieved 99.51% by nZVC of 0.5 g/L at pH of 3, open to the air, 35 °C. According to the quenching experiments and EPR spectra, HO⁻ was determined as the dominant ROS, and O₂⁻ also participated in the reaction. The conversion among Cu⁰, Cu⁺, and Cu²⁺ played a vital role for the generation of ROS. HPLC-TOF-MS was employed for the determination of ENR degradation products, the results revealed that the
degradation of ENR could be mainly achieved through decarboxylation, deacetylation and cleavage of quinolinone and piperazine rings, then the quinolone ring was opened and underwent further oxidation, deamination, and dehydrogenation to achieve complete mineralization of ENR. The obtained results deepened our understanding of the destruction of ENR with molecular oxygen activated by nZVC, and proposed a capable and practical approach for ENR removal.

**Declarations**

**Data availability**

All data generated and analyzed in this study are available upon reasonable request. Access to data generated in this report should be sent to the corresponding author at gjh@yfi.ac.cn.

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**Contributions**

Zhiqiang Gong: Data curation, Writing - original draft; Junpu Xie: Data curation, Writing-Original draft preparation; Jingxin Liu: Data curation; Ting Liu: Writing - review&editing; Jianwu Chen: Methodology, Validation; Jinping Li: Supervision; Jinhua Gan: Conceptualization, Methodology, Software Supervision.

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Declaration of Competing Interest**

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

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Tables

Table 1 is available in the Supplemental Files section.

Figures

**Figure 1**

SEM images of nZVC in different sizes: (a) 2μm; (b) 500nm; (c) 100nm; (d) EDS dot sweep of nZVC; (e) Size distribution of nZVC.
Figure 2

(a) XRD patterns of nZVC powders; (b) ENR removal in different systems: [ENR]=10 mg/L, [nZVC]=0.5 g/L, T=25℃, initial pH=3; (c) Variation of H₂O₂ and Cu⁺ with the reaction time in an nZVC/O₂ system: [ENR]=10 mg/L, [nZVC]=0.5 g/L, initial pH=3, T=25℃, t=0-60 min; (d) XPS spectrum of Cu after reaction; (e) Identification of ROS in the nZVC/O₂ system, effects of different scavengers on the degradation of ENR: [ENR]=10 mg/L, [nZVC]=0.5 g/L, [TBA]/[p-BQ] = 50 mmol/L, T=25℃, initial pH = 3; (f) EPR spectra for DMPO-HO• adducts; (g) EPR spectra for DMPO- adducts.

Figure 3
Effect of experimental conditions on ENR degradation in nZVC/O₂ system: effect of initial pH (a) and Cu leaching (b) (experimental condition: [ENR]=10 mg/L, [nZVC]=0.5 g/L, T=25°C); effect of nZVC dosage (c and d) (experimental condition: [ENR]=10 mg/L, T=25°C, initial pH=3).

Figure 4

Effect of temperature (a and b) (experimental condition: [ENR]=10 mg/L, [nZVC]=0.5 g/L, initial pH=3); (c) Effects of coexisting anions and humic acid on ENR degradation in nZVC/O₂ system (experimental condition: [ENR]=10 mg/L, [nZVC]=0.5 g/L, initial pH=3, T=25°C, t=60 min).

Figure 5
Proposed reaction pathway for ENR degradation with nZVC/O$_2$ system

Supplementary Files

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