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Original Research

Degradation of Levofloxacin via Fenton Oxidation Combined with Ultrasonic Treatment in Water

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Abstract

Levofloxacin, as a typical fluoroquinolone antibiotic, is widely used in human bacterial infection treatment and animal husbandry. However, conventional treatment technologies (e.g., precipitation, coagulation, and microbial metabolism) have limited ability to remove levofloxacin from wastewater. In this work, Fenton oxidation combined with ultrasonic treatment was investigated to efficiently remove levofloxacin from water. As a result, the optimal conditions for Fenton oxidation were first determined by the design of an orthogonal experiment. Then the combined effect of Fenton oxidation and ultrasonic treatment demonstrated a positive synergistic effect. In terms of the sequence selection of Fenton oxidation and ultrasonic treatment, Fenton–ultrasonic (10 min)–interval (40 min) was the best process. Finally, a total of 12 intermediates of levofloxacin were identified via HPLC-MS spectra, and possible degradation pathways were tentatively inferred. In addition, the toxicities of the intermediates were estimated using the Toxicity Estimation Software Tool according to the U.S. EPA proposed standards, and the results suggest that the overall toxicities of the intermediates were relatively alleviated in comparison with levofloxacin. This study provides a strategy for improving the degradation of levofloxacin via Fenton oxidation combined with ultrasonic treatment with a positive synergistic effect in water.

Keywords: levofloxacin, Fenton oxidation, ultrasonic

Introduction

Fluoroquinolone antibiotics have been widely used in the treatment of human bacterial infections [1] and veterinary medicine [2] because of their broad-spectrum and high-efficiency antibacterial properties. However, the widespread use of antibiotics increases the drug resistance of sensitive bacteria [3] and contributes to the continuous accumulation of drugs in the environment [4]. Undigested antibiotics have been frequently detected in various aquatic environments worldwide. For instance, the total usage of 36 antibiotics in China was 92,700 tons in 2013 after various wastewater tests [5], and an estimated 53,800 tons of undigested antibiotics entered the environment, with antibiotic contamination of water and soil accounting for 46% and 54%, respectively. For sewage treatment plants in the U.K., the effluent concentrations at a single sewage plant varied by up to 10-fold for levofloxacin over four years from 2012 to 2015 [6]. The indiscriminate overuse

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and misuse of antibiotics pose threats to human health and aquatic ecosystems.

Levofloxacin is one of the most frequently used fluoroquinolone antibiotics as a member of the third-generation fluoroquinolones [7]. Due to its refractory and chemical stability, levofloxacin in water is difficult to remove in conventional sewage treatment processes such as precipitation, coagulation, and microbial metabolism in wastewater treatment plants [8]. Until now, many approaches have been extensively used in the treatment of refractory antibiotic wastewater, such as adsorption [9], ultrasonic technology [10], photocatalysis [11], Fenton oxidation [12], and other advanced oxidation processes [13]. Recently, Fenton oxidation has been widely applied to levofloxacin degradation due to it being an easy method requiring simple conditions. As shown in Equations (1)-(2), the mechanism of the Fenton reaction is that hydrogen peroxide (H_2O_2) decomposes to produce hydroxyl radical (•OH) through the catalysis of ferrous acid [14]. The degradation efficiency of H_2O_2/Fe^{2+} is significantly higher than that of $H_2O_2/S_2O_8^{2-}/Fe^{2+}$ and $S_2O_8^{2-}/Fe^{2+}$ [12], and •OH is the predominant radical in the Fenton system.

$$Fe^{2+} + H_2O_2 + H^+ \rightarrow Fe^{3+} + \bullet OH + H_2O \quad (1)$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + H^+ + HO_2\bullet \quad (2)$$

Ultrasonic degradation is also considered an advanced strategy for eliminating harmful organic pollutants in wastewater. Ultrasonic waves create an extreme environment of high temperature and pressure in the solution through the cavitation effect, stimulating the formation of radicals and promoting redox reactions [15]. This reduces the complexation of hole electron pairs and promotes the production of strong oxidizing radicals [16]. For instance, Fan et al. [17] reported that the sono-photocatalytic efficiency of the $FeVO_4/BiVO_4$ heterojunction catalyst was better than that of single sonocatalysis or photocatalysis for levofloxacin degradation. A previous study showed that sonocatalysis can eradicate tetracycline hydrochloride with a $CoFe_2O_4/g\text{-}C_3N_4$ composite [18]. Zhang et al. [19] also found that the sonocatalysis method could enhance the efficiency of removing objectionable pollutants by using organic–inorganic nanocomposites (Fe_3O_4@MIL-100(Fe)/ZnS) in water. Ultrasonic and sludge biochar showed a significant synergistic enhancement in periodate activation compared with a single periodate activation system [20]. Although many studies have reported that levofloxacin can be efficiently removed from water, no previous report has investigated levofloxacin degradation via Fenton oxidation with the assistance of ultrasonic degradation in water.

Therefore, the objectives of this study were (1) to determine the optimal conditions of Fenton oxidation through an orthogonal experimental design; (2) to analyze the combined effect of Fenton oxidation and ultrasonic treatment; and (3) to speculate on the possible degradation pathways and toxicity by exploring the degradation intermediates.

Material and Methods

Chemicals and Materials

Solid levofloxacin (98%) was purchased from Shanghai Maclin Technology Co., Ltd. (Shanghai, China). Ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$) was obtained from Tianjin Bodi Chemical Co., Ltd. (Tianjin, China). Hydrogen peroxide (H_2O_2, 30%), hydrochloric acid (HCl), and formic acid were supplied by Tianjin Kemi Ou Chemical Reagent Co., Ltd. (Tianjin, China). Chromatographic grade acetonitrile was purchased from Oceanpak (Sweden). Deionized water was used to prepare an aqueous solution for the experiment.

Levofloxacin Degradation Test

Fenton's reagents consisted of H_2O_2 and $FeSO_4$. The Fenton reaction was first carried out under different conditions to select the optimal pH value, initial concentration of H_2O_2, concentration of Fe^{2+}, and reaction time. The initial concentration of levofloxacin was 20 mg·L^{-1} and the stirring time was 4 min for Fenton oxidation (n = 3). The single-factor effects involved four aspects, including (A) pH (Fe^{2+} = 0.2 mmol·L^{-1}, H_2O_2 = 1.3 mmol·L^{-1}), (B) H_2O_2 concentrations (pH = 5, Fe^{2+} = 0.2 mmol·L^{-1}), (C) Fe^{2+} concentrations (pH = 5, H_2O_2 = 1.3 mmol·L^{-1}), and (D) reaction time (pH = 5, Fe^{2+} = 0.2 mmol·L^{-1}, H_2O_2 = 1.3 mmol·L^{-1}), respectively.

According to the above optimal results of single-factor conditions, an orthogonal experiment was designed with four factors and three levels (L_9 (3^4)) to optimize the conditions of Fenton oxidation. Fenton's reagents were added to 300 mL of 20 mg·L^{-1} levofloxacin in aqueous solution under stirring. The conditions of the Fenton reaction were set as follows: pH value was 5, Fe^{2+} was 0.17 mmol·L^{-1}, H_2O_2 was 1.3 mmol·L^{-1}, and the stirring time was 4 min.

Moreover, an ultrasound instrument (JP-100S, 40 KHz, 600 W) was obtained from Shenzhen Jie Meng Equipment Co., Ltd. (Shenzhen, China) to conduct the degradation of levofloxacin. When Fenton oxidation was combined with ultrasonic treatment, the action time of ultrasonic was selected as 10 min at 600 W, and the conditions of Fenton oxidation were selected as the optimal results of the above orthogonal experiment. At a given interval, 5 mL of the suspension was retrieved and immediately mixed with 0.5 mL of ethanol, allowing it to terminate the degradation reaction. The concentration of residual levofloxacin in the supernatant was determined via high performance liquid chromatography (HPLC) equipped with a UV detector

(Agress 1100, Dalian Elite Analytical Instruments Co., Ltd., China) [21]. A reverse phase C18 column (150 mm × 4.6 mm × 5 μm) was used for the separation. Methanol and ammonium acetate (70:30, v/v) were used as the mobile phase, and the flow rate was set at 1.2 $mL \cdot min^{-1}$. A linear range of the standard curve was set to 5-50 $mg \cdot L^{-1}$, and the correlation coefficient was 0.998.

In addition, the contents of H_2O_2 were spectrophotometrically determined using the metavanadate method [22]. The levels of iron (II) and iron (III) were determined using the spectrophotometric method with 1,10-phenanthroline [23]. The mineralization rate was expressed by the removal rate of the total organic carbon (TOC) in the water, and the contents of TOC were determined using the methods of combustion oxidation and non-dispersive infrared spectrometry [24].

Qualitative Analysis of Degradation Products

The intermediates of levofloxacin degradation were qualitatively analyzed using high-performance liquid chromatography-mass spectrometry (HPLC-MS). Agilent 1100 LC and Thermo Scientific TSQ Quantum Ultra AM were equipped with a mass-charge ratio (*m/z*) scanning range of 40-400. A Welch Ultimate XB-C18 column (2.1×100 mm, 3 μm) was used for chemical separation. The mobile phase consisted of water (0.1% formic acid) and acetonitrile. An amount of 8% acetonitrile was used for 9 minutes at the beginning, and then its concentration was increased from 8% to 92% for 10.50 min, and finally, the volume of acetonitrile was adjusted to the original 8% after 10.51 min. The flow rate was set at 0.3 $mL \cdot min^{-1,}$ and the injection size of the sample was 10 μL. The conditions for MS were set as follows: ion source: HESI II, spray voltage: 3300 V, heater temperature: 450ºC, sheath gas pressure: 42 Arb, ion sweep gas pressure: 1, aux gas pressure: 13 Arb, capillary temperature: 350ºC, tube lens offset: –19 V, and in-source CID: 0 V.

Toxicity Assessment

The toxicities of levofloxacin and its degradation intermediates were estimated using the Toxicity Estimation Software Tool (TEST) according to the U.S. EPA proposed standards [25].

Statistical Analysis

Statistical differences were determined using an analysis of variance, and multiple comparisons were analyzed using the Tukey test. The interaction between the Fenton-ultrasonic treatment and the ultrasonic treatment was performed using an analysis of variance of the two-factor repeated design using SPSS 22.0.

Results and Discussion

Optimization of Fenton Reaction Conditions

Effect of Solution pH

As shown in Fig. 1a), the trend of the pH effect increased first and then decreased, reaching its maximum value when the pH was 4. The degradable efficiency of levofloxacin in Fenton oxidation decreased when the solution had a higher pH value. The reason for this phenomenon was that Fe^{2+} and Fe^{3+} can generate precipitation and flocculation under alkaline conditions, thus preventing the formation of •OH [26]. Therefore, the pH value was adjusted to 4 for the subsequent Fenton reaction.

Effect of H_2O_2 Concentration

According to the principle of reaction, the dosage of H_2O_2 determined the effectiveness and economy of the Fenton oxidation system; therefore, it was necessary to investigate the effect of the dosage of H_2O_2 on the levofloxacin removal rate. As shown in Fig. 1b), when the dosages of H_2O_2 were in the range of 0.65-2.30 $mmol \cdot L^{-1}$, the degradation rates of levofloxacin increased with the increase in the dosage of H_2O_2. But when the dosage of H_2O_2 was greater than 2.30 $mmol \cdot L^{-1}$, the degradation rates of levofloxacin in water gradually decreased. Under the condition of low H_2O_2 concentrations, the amount of •OH was increased with the increase in H_2O_2 concentrations, and the oxidation efficiencies of organic matter were gradually strengthened. However, as shown in Fig. S1 in the supplementary material (SM), the levels of residual H_2O_2 in water did not proportionally increase with the gradual increase of H_2O_2 concentrations. Because HO_2• was generated through the reaction between H_2O_2 and •OH (Equation 3), it resulted in a decrease in the contents of •OH [13], which also affected the oxidation efficiency of levofloxacin. Therefore, the concentration of H_2O_2 should be based on an appropriate range; in the case of a high dose, not only would it increase the consumption of H_2O_2, but it would also affect the degradation rates of levofloxacin. Therefore, an H_2O_2 amount of 1.3 $mmol \cdot L^{-1}$ was the optimal dosage to remove levofloxacin in this Fenton oxidation system.

$$H_2O_2 + \bullet OH \rightarrow HO_2\bullet + H_2O \quad (3)$$

Effect of Fe^{2+} Concentration

The effect of the dosage of Fe^{2+} on the removal of levofloxacin is shown in Fig. 1c). When the dosage of Fe^{2+} ranged from 0.16 to 0.33 $mmol \cdot L^{-1}$, the degradation rates of levofloxacin initially increased and then decreased, reaching the highest value when the concentration was 0.22 $mmol \cdot L^{-1}$. Apparently,

when the dosage of Fe^{2+} reached an excessive level, precipitation and flocculation occurred [27], and the degradation rate began to decline. Therefore, an Fe^{2+} amount of 0.22 mmol·L^{-1} is the optimal dosage for levofloxacin degradation in this Fenton oxidation system.

Effect of Reaction Time

As shown in Fig. 1d), the degradation rates of levofloxacin increased rapidly within 40 minutes, and the removal rate of levofloxacin reached 81.48%, while the time effect curve of levofloxacin degradation tended to be flat after 40 minutes. And the contents of residual H_2O_2 in the water were in the range of 5% to 10% from 40 min to 80 min, compared with the added amounts of H_2O_2 (Fig. S2). A possible explanation was that, in the early stage of Fenton oxidation, the H_2O_2 decomposed a large amount of •OH under the catalysis of sufficient Fe^{2+}, which rapidly attacked the levofloxacin and destroyed its molecular structure. However, as the reaction continued, Fe^{2+} was gradually converted to Fe^{3+}, reducing the content of •OH [28]. At the same time, the oxidative decomposition process of levofloxacin produced a variety of intermediates, which may have competed with levofloxacin, slowing down its degradation rate. Therefore, the optimal reaction time was 40 minutes in the following Fenton reaction.

In summary, the results of the single-factor optimization were listed as follows: The pH was 4, the concentration of hydrogen peroxide was 1.5 mmol·L^{-1}, the concentration of iron was 0.22 mmol·L^{-1}, and the reaction time was 40 minutes. Based on these results, an orthogonal experiment was designed to further optimize the Fenton oxidation system in water. Four factors and three levels (L_9 (3^4)) were selected for the orthogonal experimental design; the four factors were pH, initial concentrations of hydrogen peroxide and ferric ion, and stirring time, and their three relevant levels are presented in Table S1 in the SM.

Results of the Orthogonal Test of Levofloxacin Degradation via Fenton Oxidation

The Fenton reagent is highly oxidizing because H_2O_2 can be catalyzed by Fe^{2+} to produce strongly oxidizing

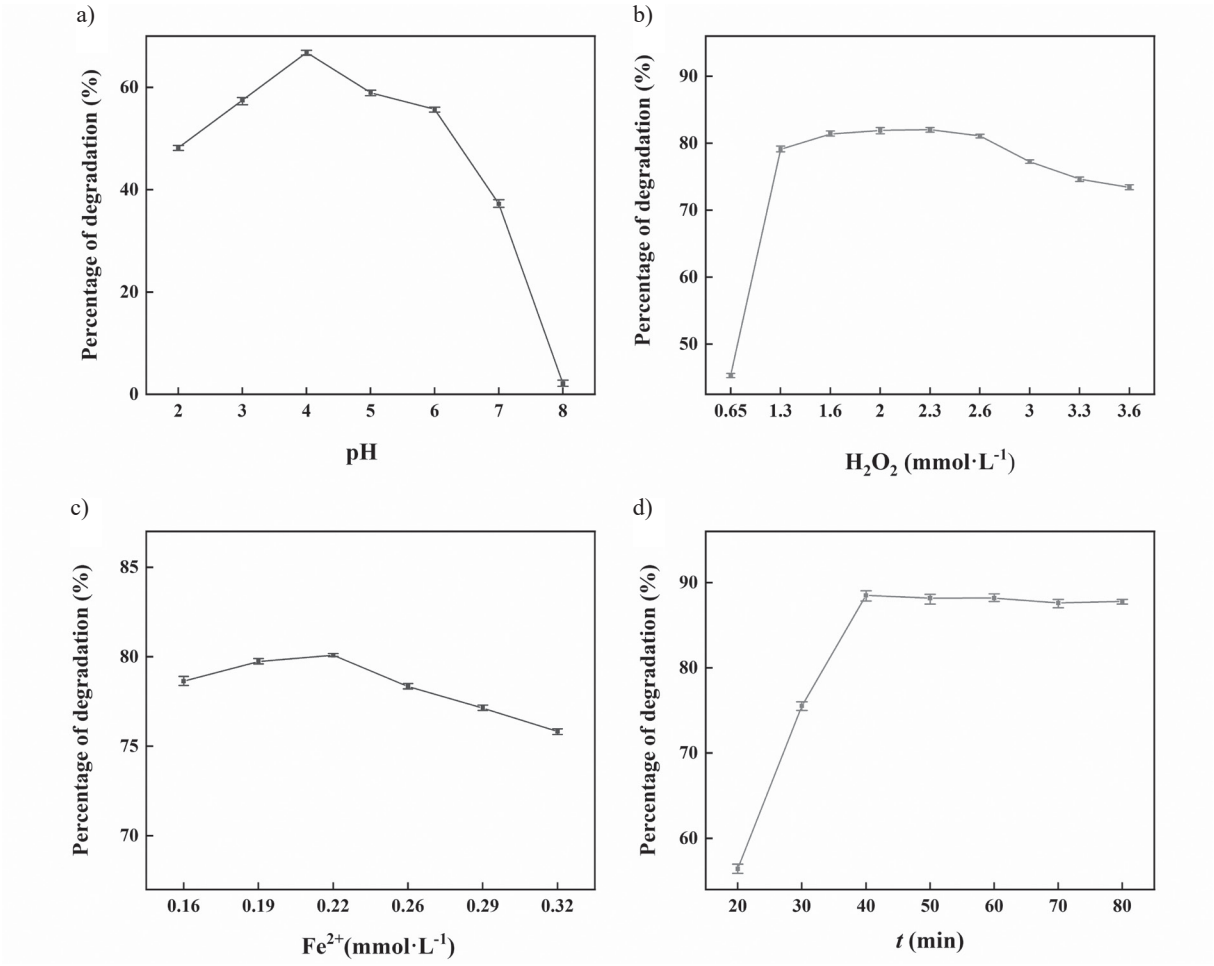

Fig. 1. The effects of a) pH (Fe^{2+} = 0.2 mmol·L^{-1}, H_2O_2 = 1.3 mmol·L^{-1}), b) H_2O_2 concentrations (pH = 5, Fe^{2+} = 0.2 mmol·L^{-1}), c) Fe^{2+} concentrations (pH = 5, H_2O_2 = 1.3 mmol·L^{-1}), and d) reaction time (pH = 5, Fe^{2+} = 0.2 mmol·L^{-1}, H_2O_2 = 1.3 mmol·L^{-1}) on the degradation of (initial concentration = 20 mg·L^{-1}) via Fenton oxidation in water (n = 3).

•OH; therefore, the molar ratio of H_2O_2/Fe^{2+} would also affect the oxidation efficiency. But the yield of •OH was closely related to the experimental conditions, such as the initial pH of the reaction system, the initial concentration of H_2O_2, the molar ratio of Fe^{2+} to H_2O_2, and the reaction time.

The results of an orthogonal test of levofloxacin degradation via Fenton oxidation are shown in Table S2 in the SM, and the results of the visual analysis are presented in Table 1. The effects of four factors on the degradation of levofloxacin via Fenton oxidation followed a descending trend of pH>molar ratio of H_2O_2/Fe^{2+}>initial concentration of H_2O_2>stirring time. The optimal reaction condition combination was $A_2B_3C_3D_3$, that is, the initial pH value was 5, the molar ratio of H_2O_2/Fe^{2+} was 7.5:1, the initial concentration of H_2O_2 was 1.3 mmol·L^{-1}, and the stirring time was 4 min. Therefore, these optimal conditions were carried out in the following Fenton reaction.

Treatment of Levofloxacin in Water via Fenton Oxidation Combined with Ultrasonic Treatment

The Sequence Selection between Fenton Oxidation and Ultrasonic Treatment

In order to optimize the combination method with the highest degradation rate between Fenton oxidation and ultrasonic degradation, three different treatments were carried out as follows: (A) Fenton reagent was added first, then the interval was 40 minutes, and finally the ultrasonic treatment was 10 minutes (Fenton-interval (40 min) -ultrasonic); (B) the Fenton reagent was added first, followed by ultrasonic treatment for 10 min, and finally the interval was 40 minutes (Fenton–ultrasonic–interval (40 min)); and (C) the ultrasonic treatment was firstly performed for 10 min, followed by the addition of Fenton reagent, and finally the interval was 40 minutes (ultrasonic (10 min)–Fenton–interval (40 min)).

For the combination of Fenton and ultrasonics, the degradation rate of levofloxacin in water increased gradually with the increase in time during the 40 minutes. As shown in Fig. S3, method B, Fenton–ultrasonic–interval (40 min), had the highest degradation rates of levofloxacin in water. The ultrasonic treatment sped up the conversion of Fe^{3+} to Fe^{2+}; therefore, it accelerated the regeneration of the catalyst Fe^{2+} and then catalyzed the pyrolysis of H_2O_2 to produce more •OH. This solved the shortcomings of Fe^{2+} in the traditional Fenton oxidation process, which was quickly consumed and not easy to regenerate. In addition, the cavitation bubble generated by ultrasonic waves in the liquid phase is an extreme physical microenvironment that can promote the thermal cracking of H_2O_2 to generate •OH, which is released into the surrounding environment with the rupture of the cavitation bubble [29]. Therefore, the intervention of the ultrasonic treatment provides a new method for the generation of •OH via the Fenton oxidation process.

Table 1. The results of the orthogonal test and visual analysis of levofloxacin degradation via Fenton oxidation in water.

	A (pH)	B (H_2O_2) (mmol·L^{-1})	C (H_2O_2/Fe^{2+}) (molar ratio)	D (t) (min)	Degradation rates (%)
1	4	0.65	4.5:1	2	28.34
2	4	0.98	5:1	3	18.97
3	4	1.3	7.5:1	4	67.02
4	5	0.65	5:1	4	66.84
5	5	0.98	7.5:1	2	76.89
6	5	1.3	4.5:1	3	81.98
7	6	0.65	7.5:1	3	50.07
8	6	0.98	4.5:1	4	54.78
9	6	1.3	5:1	2	49.78
K_1	114.33	145.25	165.10	155.01	
K_2	225.71	150.64	135.59	151.02	
K_3	154.63	198.78	193.98	188.64	
$\bar{K}_1$	38.11	48.42	55.03	51.67	
$\bar{K}_2$	75.24	50.21	45.20	50.34	
$\bar{K}_3$	51.54	66.26	64.66	62.88	
R	37.13	17.84	19.46	12.54	

Note: K_1–K_3 represents the sum of degradation rates at the same level; $\bar{K}_1$–$\bar{K}_3$ represents the average of degradation rates at the same level; and R represents the range of the mean.

Method B (Fig. S3), Fenton–ultrasonic–interval (40 min), was carried out in the following treatment of Fenton oxidation combined with ultrasonic degradation.

Combined Effect of Fenton Oxidation and Ultrasonic Treatment

The types of joint effects include additive, synergistic, or antagonistic effects. The types can be determined by the following strategies [30]. First, the interaction between the two treatments was judged through an analysis of variance of the two-factor repeated design. If the interaction was not statistically significant, there was an additive effect between the two treatments; if the interaction was statistically significant, it indicated that there was not an additive but a synergistic or antagonistic effect. Furthermore, the dose–effect curve was drawn to determine whether it was a synergistic or antagonistic effect. If the two curves diverged with increasing dose, it was a synergistic effect. On the contrary, if the two curves were close or crossed with the increase in dose, it was an antagonistic effect.

In the present study, the degradation rates of levofloxacin via Fenton–ultrasonic and ultrasonic treatments are presented in Table S2 and were analyzed using an analysis of variance of the two-factor repeated design (Table 2). The interaction between the Fenton–ultrasonic treatment and the ultrasonic treatment was statistically significant (p<0.01), indicating that the two treatments deviated from an additive effect. Furthermore, the degradation rates of levofloxacin via ultrasonic treatment alone, as shown in Fig. 2, were only approximately ten percent. The degradation rates of levofloxacin via Fenton oxidation combined with ultrasonic treatment were up to 96%, which is obviously better than that of the single ultrasonic treatment, and the trend of the two curves was gradually diverging. These two results indicate that Fenton combined with ultrasonic had a positive synergistic effect on levofloxacin degradation in water; therefore, ultrasonic treatment can promote the degradation efficiency of levofloxacin in water when combined with Fenton oxidation.

The synergistic effect of Fenton combined ultrasound can be achieved in the following ways: First, ultrasonic technology not only produces hydroxyl free radicals itself [16], but also promotes Fenton reagents to produce more hydroxyl radicals, accelerating the degradation of organic pollutants. Second, the pyrolysis caused by the cavitation effect of ultrasonics can speed up the reaction [15]. Third, ultrasonic plays the role of stirring and mass transfer, promoting the diffusion of reactants, accelerating the oxidation rate of the Fenton reagent, and significantly improving the degradation efficiency. In the Fenton reaction, as shown in Equations (4)-(6), the degradation of organic compounds depends on the interaction of hydroxyl radicals with organic compounds to generate small molecular organic chemicals, such as carbon dioxide and water [31].

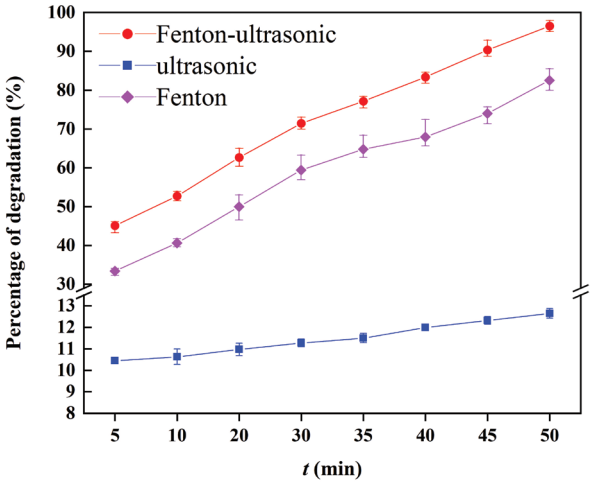

Fig. 2. Comparison of time–effect curves among the treatments of ultrasonic, Fenton, and Fenton–ultrasonic (n = 3). Ultrasonic conditions: 600 W, 10 min; Fenton reaction conditions: initial levofloxacin concentration was 20 mg·L^{-1}, pH value was 5, Fe^{2+} was 0.17 mmol·L^{-1}, H_2O_2 was 1.3 mmol·L^{-1}, and the stirring time was 4 min. Fenton-ultrasonic conditions: Fenton–ultrasonic (10 min)–interval (40 min).

$$RH + \bullet OH \rightarrow R\bullet + H_2O \quad (4)$$

$$R\bullet + Fe^{3+} \rightarrow Fe^{2+} + R^+ \quad (5)$$

$$R^+ + O_2 \rightarrow ROO^+ \rightarrow R' + CO_2 + H_2O \quad (6)$$

Table 2. Variance analysis of levofloxacin degradation in water comparing Fenton–ultrasonic to ultrasonic treatments.

Sources	*SS*	*df*	S^2	*F* test	Significance
Fenton-ultrasonic	44571.712	1	44571.712	30513.523	0.0001
Ultrasonic	3715.189	7	530.741	363.342	0.0001
Fenton-ultrasonic * Ultrasonic	3121.653	7	445.950	305.295	0.001
Error	46.743	32	1.461		
Total variation	51455.297	47			

SS: Sum of squares, *df*: degree of freedom, S^2: Squared value, *represented interaction.

Movever, the addition of iron to the Fenton reagent will increase the secondary pollution of metal. Therefore, the content of iron in water was determined. As a result, the contents of iron ions in the water were almost undetectable (below the detection limit, the data was not shown). The reason was that there was a large amount of hydrogen peroxide in the water (the mole ratio of hydrogen peroxide to iron was 7.5:1), so the iron ions (II and III) in the water were almost exhausted and eventually existed in the form of iron hydroxide in the sludge. As shown in Equations (7)-(9), the iron hydroxide colloids generated in the reaction can remove part of the organic pollutants in water through flocculation. But the disposal of iron sludge will be a thorny issue. As previously reported, Fe_2O_3, Fe_3O_4, and Fe were recovered from Fenton sludge by the calcination process, and the iron in the sludge was utilized as a resource [32, 33]. Because under different calcination temperatures, the transformation sequence of iron was $Fe(OH)_3$ or FeOOH → Fe_2O_3 → Fe_3O_4 → FeO → Fe in the Fenton sludge [33].

$$Fe^{2+} + O_2 + 2H^+ \rightarrow Fe(OH)_2 \quad (7)$$

$$4Fe(OH)_2 + O_2 + H_2O \rightarrow 4Fe(OH)_3 \quad (8)$$

$$Fe^{3+} + OH^- \rightarrow Fe(OH)_3 \quad (9)$$

In addition, to distinguish the differences among the ultrasonic, Fenton, and Fenton-ultrasonic treatments statistically, an analysis of variance was conducted by averaging degradation rates and mineralization rates among the different methods. As shown in Table 3, both the degradation rates and mineralization rates between ultrasonic and Fenton-ultrasonic showed a statistically significant difference (n = 3, p<0.05). Similarly, the degradation rates or mineralization rates in Fenton-ultrasonic were significantly higher than those of Fenton oxidation. These results indicate that significantly higher degradation rates and mineralization rates occurred in the Fenton–ultrasonic treatment.

Degradation Pathways of Levofloxacin

The intermediates during the degradation of levofloxacin in Fenton treatment combined with ultrasonic treatment were qualitatively analyzed using HPLC-MS. A total of 12 substances were detected, and their basic information is summarized in Table S3, including retention time, mass–charge ratio (*m*/*z*), and inferred structure. Their chromatograms and mass spectrograms are shown in Figure S4. According to the intermediates obtained in this experiment and reported in the literature [34-38], the possible degradation pathways to levofloxacin were inferred from the HPLC-MS spectra (Fig. 3).

There were six possible degradation pathways identified. In pathway I: P1 (*m*/*z* = 318) was generated by the removal of the carboxyl group from the pyridine ring of levofloxacin [36]. In pathway II: an F atom of levofloxacin was replaced by a hydroxyl group to form P2 (*m*/*z* = 360). Then, P3 (*m*/*z* 277) was generated from P2 (*m*/*z* 360) through the depiperazinyl ring. Finally, P7 (*m*/*z* 233) was generated from P3 (*m*/*z* 277) through decarboxylation. In pathway III: P4 (*m*/*z* 290) was generated from P2 (*m*/*z* 360) through decarboxylation and an open ring of piperazine, which was subsequently oxidized to form P6 (*m*/*z* 261). Finally, P7 (*m*/*z* 233) was produced from P6 (*m*/*z* 261) through hydrolysis. In pathway IV: the piperazine side chain in P2 was directly oxidized and decarboxylated to form P5 (*m*/*z* 274) and then converted to P3 (*m*/*z* 277) through hydrolysis, decarboxylation, and a series of valence bond breaks. P5 (*m*/*z* 274) was generated from P4 (*m*/*z* 290) through a dehydroxylated group. In pathway V: P8 (*m*/*z* 279) could be generated by removing the piperazine ring of levofloxacin [34]. Then, P9 (*m*/*z* 246) was generated from P8 (*m*/*z* 279) through defluorination and deamination. In pathway VI: P10 (*m*/*z* 218) was obtained from P8 (*m*/*z* 279) through defluorination and decarboxylation, then oxidized to form P11 (*m*/*z* 227) through oxidation, and finally converted to P12 (*m*/*z* 167) through a series of valence bond breaks and decarboxylated reactions [35]. The above results demonstrate that levofloxacin was degraded into CO_2, H_2O, F^-, NH_4^+, and 12 other intermediates via the Fenton oxidation process combined with ultrasonic treatment.

Table 3. The analysis of variance for the degradation rates (%) and mineralization rates (%) of levofloxacin among the treatment of ultrasonic, Fenton, and Fenton-ultrasonic.

	Methods	Degradation Rates (%)	$p < 0.05$	Mineralization rates (%)	$p < 0.05$
1	Ultrasonic	12.65 ± 0.092[a]	*A*[b]	6.34 ± 0.087[a]	*A*[b]
2	Fenton	82.53 ± 2.79	*B*	50.21 ± 1.63	*B*
3	Fenton-ultrasonic	96.53 ± 1.43	*C*	59.32 ± 2.31	*C*

[a] Mean ± SD, SD: Standard deviation (n = 3).

[b] Statistical differences were determined using an analysis of variance, and the multiple comparison was analyzed by the Tukey test. A different letter on the same column indicates a significant difference.

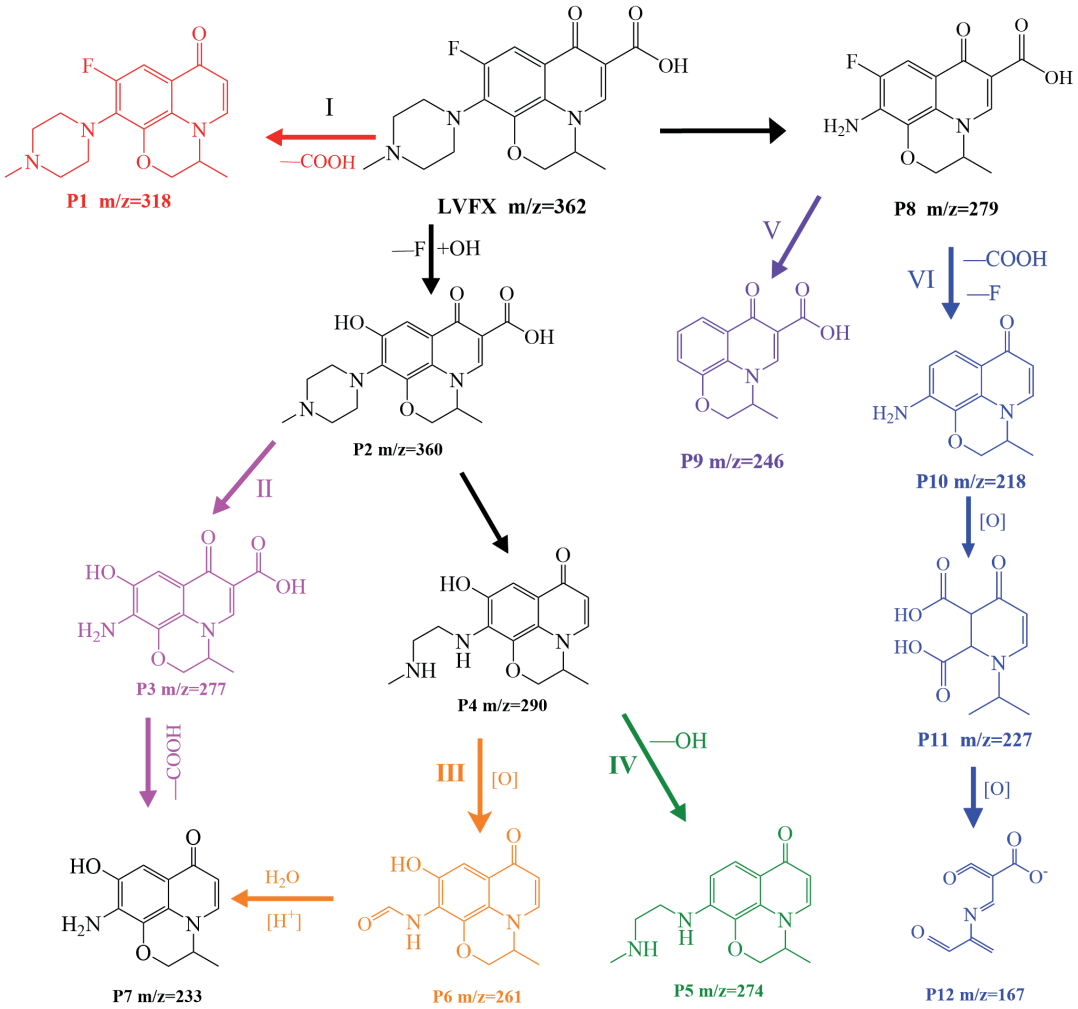

Fig. 3. The possible degradation pathways of levofloxacin during the Fenton-ultrasonic reaction in water.

Toxicity Assessment of Levofloxacin and Its Intermediates

The Toxicity Estimation Software Tool (TEST), based on the Quantitative Structure Activity Relationship (QSAR) methodologies, was used to estimate the toxicity of levofloxacin and its degradation intermediates. The toxicity endpoints included the acute toxicity of *Fathead minnow* and *Daphnia magna*, bioaccumulation factors, and developmental toxicities. First, the median lethal concentration (LC_{50}) values (96 h) of all the intermediates for *Fathead minnow* (Fig. 4a) were higher than those of levofloxacin. As previously reported [39, 40], all the toxicities of intermediates decreased compared with those of levofloxacin. Second, the LC_{50} values (48 h) of the other intermediates for *Daphnia magna* were lower than those of levofloxacin except for P1 (m/z = 318) (Fig. 4b). These results are in accordance with the literature reporting that most intermediates are harmful (10-100 $mg \cdot L^{-1}$) rather than toxic (1-10 $mg \cdot L^{-1}$) [40]. Third, except for P2 (m/z = 360) and P4 (m/z = 290), the bioaccumulation factors of the other intermediates were lower than those of levofloxacin (Fig. 4c). A previous study also showed that the bioaccumulation effect was weak for the degradation products [41]. Finally, the estimated developmental toxicities of degradation products were all lower than those of levofloxacin (Fig. 4d), which suggests that the toxicities of intermediates gradually decreased with the Fonton-ultrasonic reaction. A previous study also reported that the developmental toxicity of levofloxacin degradation products is higher than that of levofloxacin itself [42]. Overall, the estimated toxicities of intermediates were effectively alleviated in comparison with levofloxacin after degradation.

Levofloxacin, as a typical fluoroquinolone antibiotic, is widely used in human bacterial infection treatment and animal husbandry. However, conventional treatment technologies (e.g., precipitation, coagulation, and microbial metabolism) have limited ability to remove levofloxacin from wastewater. In this work, Fenton oxidation combined with ultrasonics had a synergistic effect, which improved the degradation rate of levofloxacin in water. Levofloxacin was decomposed into CO_2, H_2O, F^-, NH_4^+, and the other 12 intermediates.

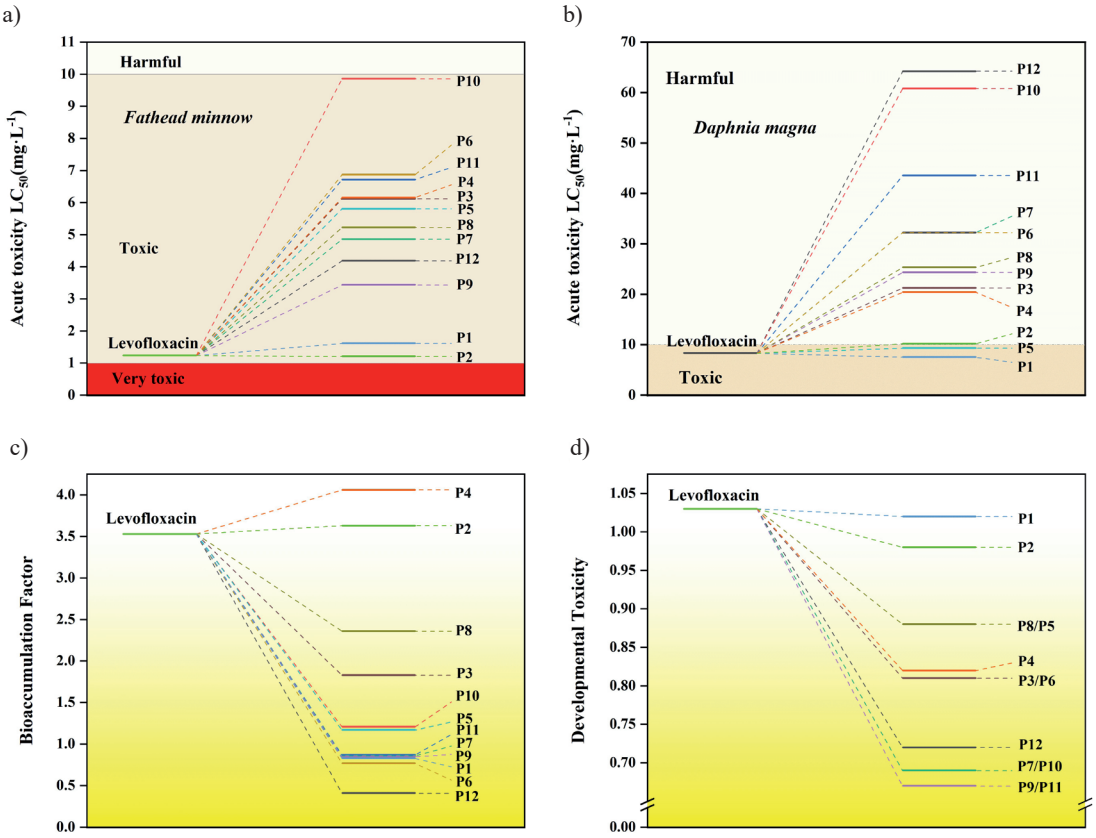

Fig. 4. The estimated toxicities of levofloxacin and its degradation intermediates. a) LC_{50} of Fathead minnow, b) LC_{50} of *Daphnia magna*, c) bioaccumulation factor, and d) developmental toxicity.

And the estimated toxicities of the intermediates were relatively alleviated in comparison with levofloxacin after degradation.

Limitations

Although Fenton and ultrasonic processes were shown to be mature and stable in actual wastewater treatment [43, 44], their mineralization rates were not the highest compared with other processes. Moreover, although the content of levofloxacin itself was reduced, a variety of intermediates were generated, which were not completely degraded into carbon dioxide and water. Third, as previously reported [45], the optimal conditions in the laboratory have a certain practical guiding significance, but they should be optimized in the pilot plant test to guide the engineering application, and the result will be more accurate in the actual water treatment. Apart from Fenton oxidation and ultrasonic degradation, adsorption and photocatalytic degradation would be the most tested techniques [46]. Other emerging AOP technologies are also promising, such as electrochemical advanced oxidation [47], sulfate radical oxidation, and heterogeneous semiconductor photocatalysis [27, 48]. However, these methods require more in-depth research to determine their technical and economic viability in the future.

Conclusions

Firstly, the optimal parameters of levofloxacin degradation via Fenton oxidation were determined through single-factor and orthogonal experiment designs. The optimum conditions are as follows: The initial pH value was 5, the molar ratio of H_2O_2/Fe^{2+} was 10:1, the initial concentration of H_2O_2 was 1.3 mmol·L^{-1}, and the stirring time was 4 min. In terms of the sequence selection of Fenton oxidation and ultrasonic treatment, Fenton–ultrasonic–left (40 min) was the best process. Secondly, the interaction between ultrasonic and Fenton–ultrasonic treatment was found to be statistically significant using the two-factor analysis of variance, and the trend of the two curves was gradually diverging. The results of the above two aspects indicate that the type of joint effect was a synergistic effect. Finally, the intermediates of levofloxacin degradation were qualitatively analyzed via HPLC-MS, and the possible degradation pathways and toxicity of levofloxacin were estimated tentatively.

Supplementary Information

The online version contains supplementary material available at https://doi.org/xxx.

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Conflict of Interests

The authors declare no conflict interest.

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Supplementary Materials

Table S1. Orthogonal experimental design (four factors and three levels) of levofloxacin degradation via Fenton oxidation in water.

	Factors	Levels		
		1	2	3
A	pH	4	5	6
B	H_2O_2 (mmol·L^{-1})	0.65	0.98	1.3
C	H_2O_2/Fe^{2+}	4.5:1	5:1	7.5:1
D	Stirring time (min)	2	3	4

Table S2. The percentage of levofloxacin degradation by ultrasonic, Fenton, and Fenton-ultrasonic treatments in water (%, $n = 3$).

t (min)	Ultrasonic			Fenton			Fenton-ultrasonic		
	1	2	3	1	2	3	1	2	3
5	10.42	10.48	10.45	32.30	34.13	33.90	43.30	46.13	45.90
10	10.56	10.79	10.53	39.70	41.78	40.50	51.50	52.78	53.90
20	10.92	11.11	10.9	46.58	53.03	50.40	62.58	65.03	60.40
30	11.27	11.35	11.2	58.07	63.27	57.00	73.07	71.27	70.00
35	11.41	11.59	11.5	62.67	68.39	63.38	77.67	75.39	78.38
40	11.98	11.98	12.03	65.76	72.45	65.70	81.76	84.65	83.70
45	12.26	12.3	12.4	71.42	74.90	75.73	89.42	92.90	88.73
50	12.54	12.7	12.7	85.50	82.11	79.97	96.50	95.11	97.97

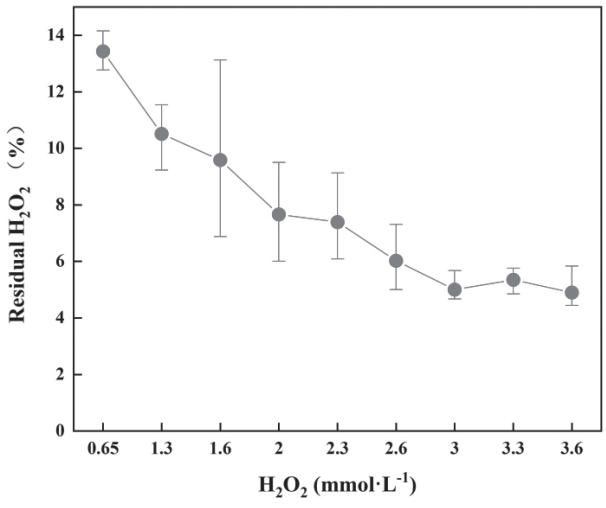


Fig. S1. The levels of residual H_2O_2 with the gradual increase of H_2O_2 concentrations in Fenton reaction.

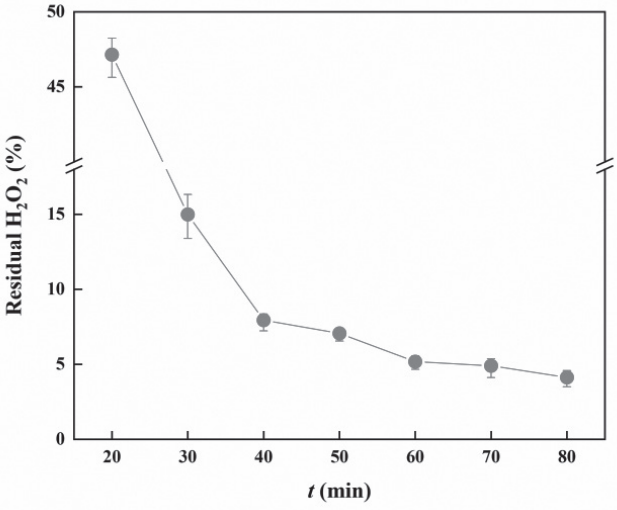
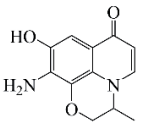
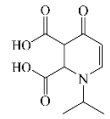
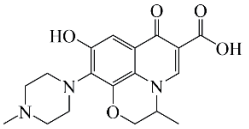
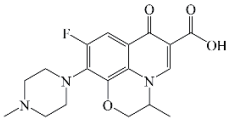
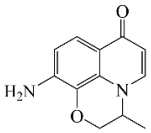
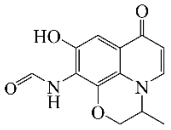
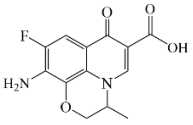
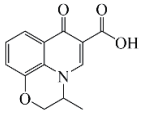
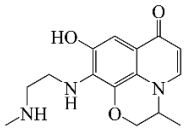
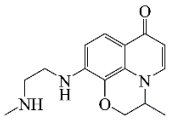
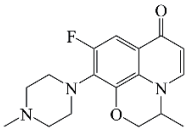
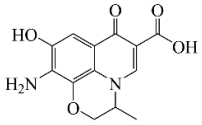
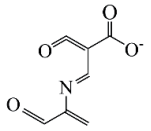


Fig. S2. The levels of residual H_2O_2 with the gradual increase of reaction time in Fenton reaction

Table S3. Proposed byproducts of levofloxacin degradation by Fenton-ultrasonics oxidation.

	RT (min)	Name	*m/z*	Molecular formula	Proposed structure
1	1.18	P7	233	$C_{12}H_{13}N_2O_3$	
2	1.43	P11	227	$C_{10}H_{13}NO_5$	
3	2.42	P2	360	$C_{18}H_{21}N_3O_5$	
4	3.66	LVFX	362	$C_{18}H_{20}FN_3O_4$	
5	5.95	P10	218	$C_{12}H_{12}N_2O_2$	
6	6.02	P6	261	$C_{13}H_{12}N_2O_4$	
7	6.19	P8	279	$C_{13}H_{11}FN_2O_4$	
8	6.99	P9	246	$C_{13}H_{11}NO_4$	
9	7.04	P4	290	$C_{15}H_{19}N_3O_3$	
10	8.07	P5	274	$C_{15}H_{19}N_3O_2$	
11	8.19	P1	318	$C_{17}H_{20}FN_3O_2$	
12	11.71	P3	277	$C_{13}H_{12}N_2O_5$	
13	13.45	P12	167	$C_7H_5NO_4$	

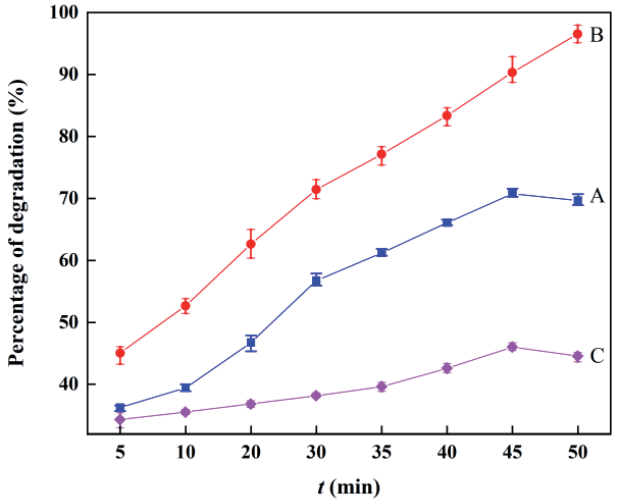


Fig. S3. Degradation curve of levofloxacin in water via (A) Fenton–interval (40 min)–ultrasonic (10 min), (B) Fenton–ultrasonic (10 min)–interval (40 min), and (C) ultrasonic (10 min) –Fenton–interval (40 min) (n = 3). Ultrasonic conditions: 600 W, 10 min; Fenton reaction conditions: initial levofloxacin concentration was 20 mg·L^{-1}, pH value was 5, Fe^{2+} was 0.17 mmol·L^{-1}, H_2O_2 was 1.3 mmol·L^{-1}, and the stirring time was 4 min.

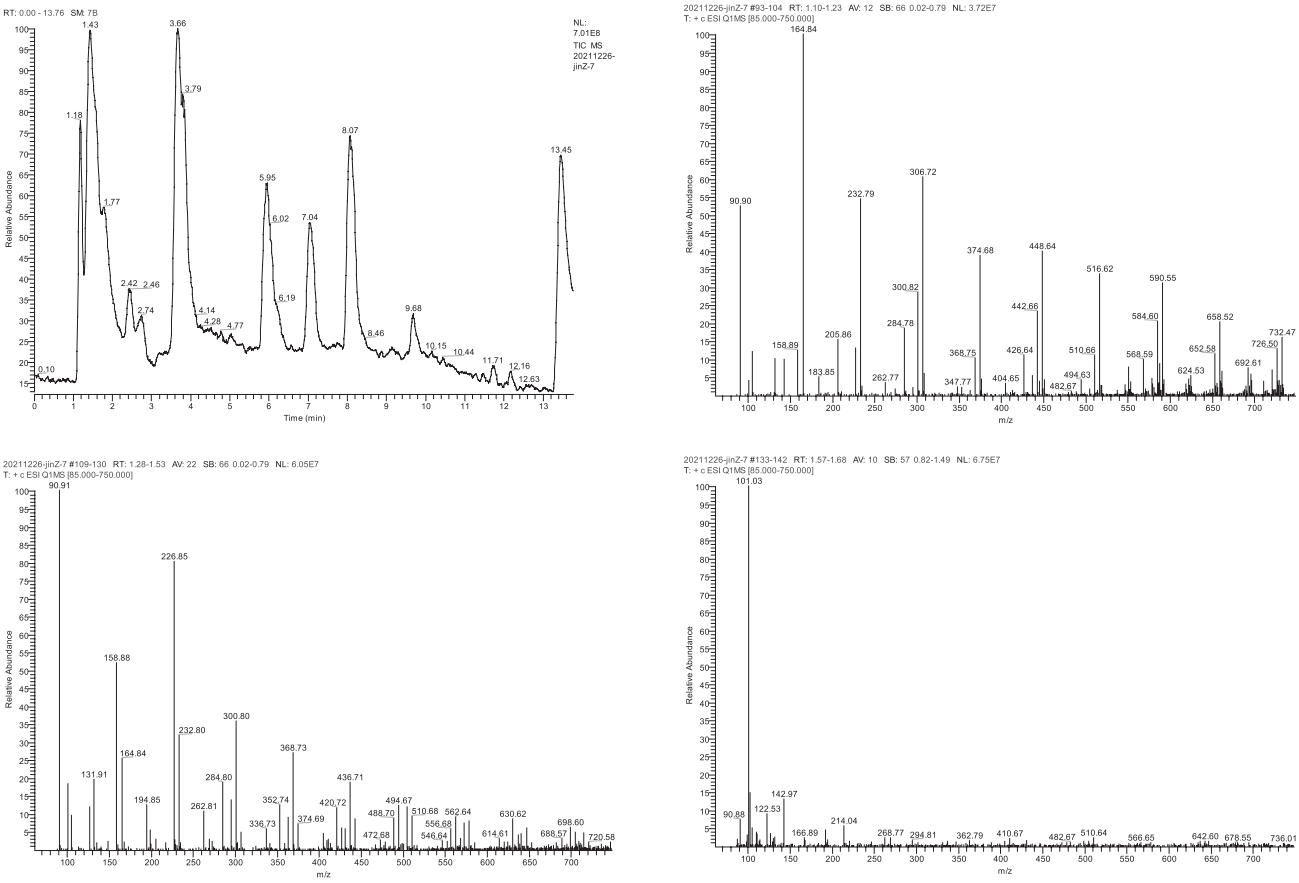


Fig. S4. Chromatograms and their mass spectrograms of the degradation byproducts of levofloxacin by Fenton-ultrasonics oxidation.

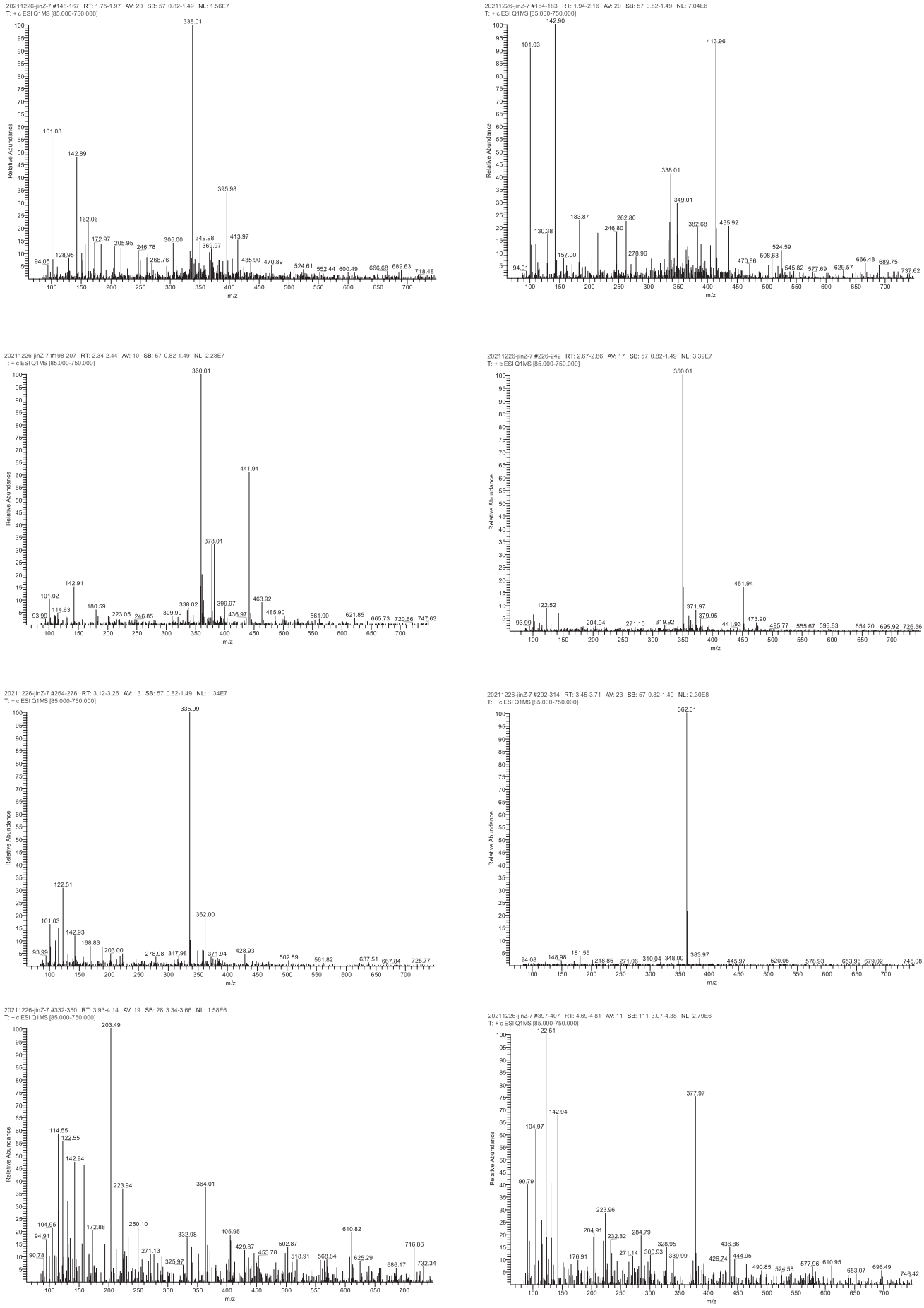
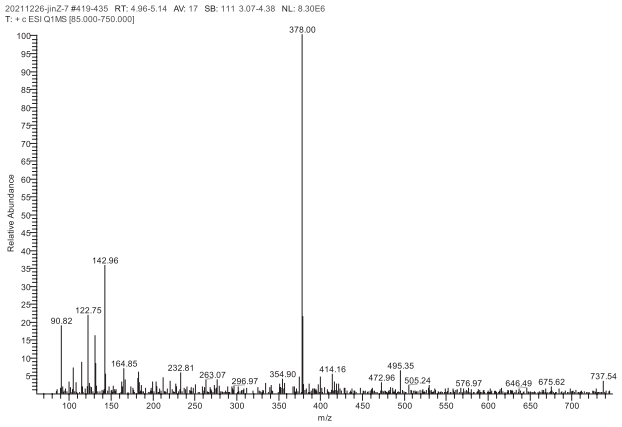
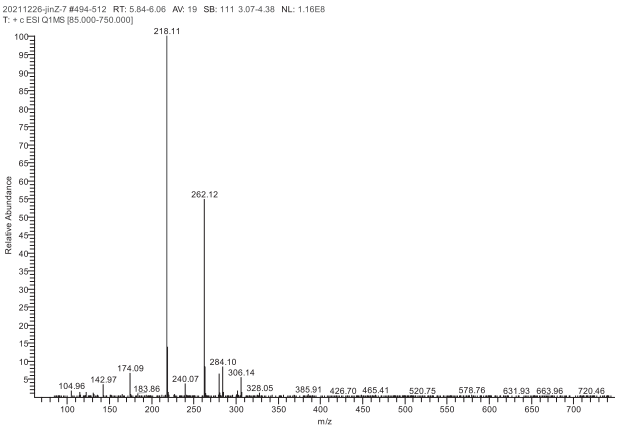
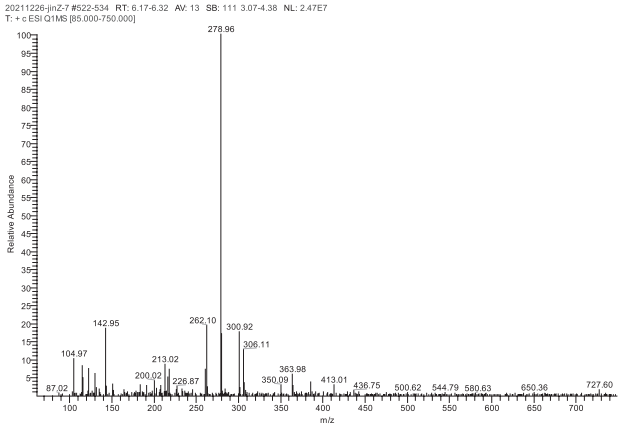
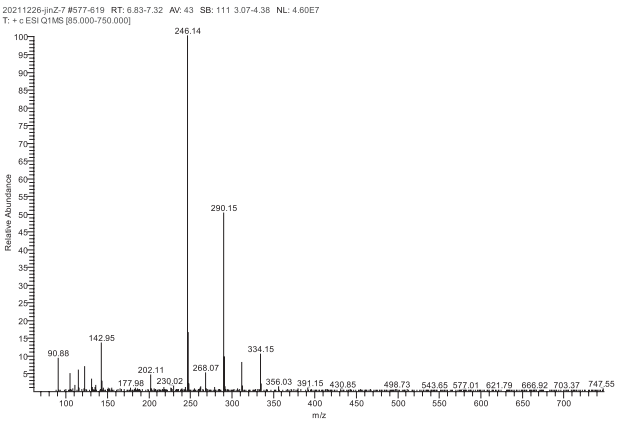


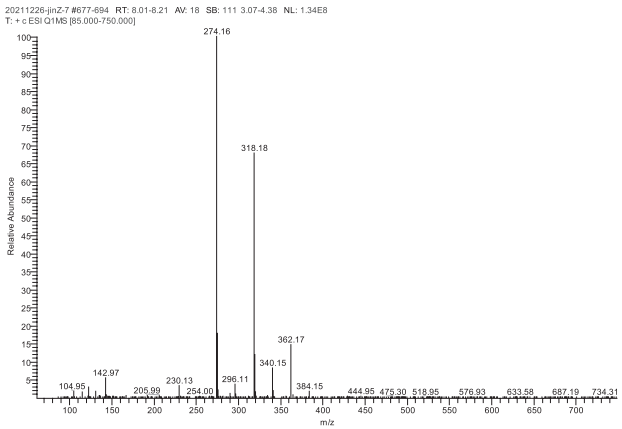
Fig. S4. Continued.

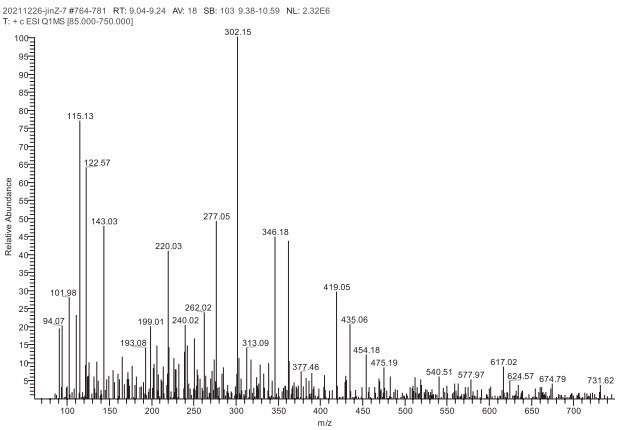


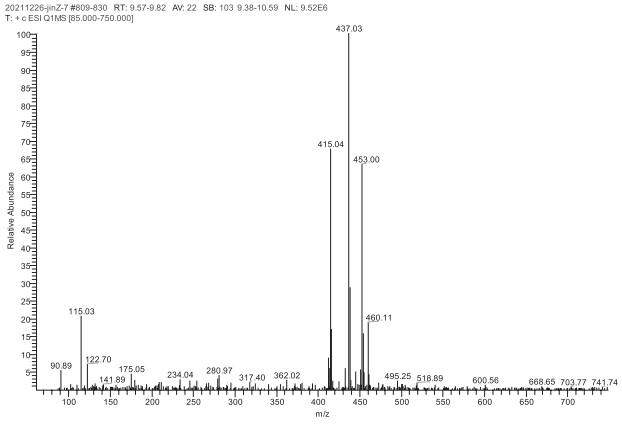












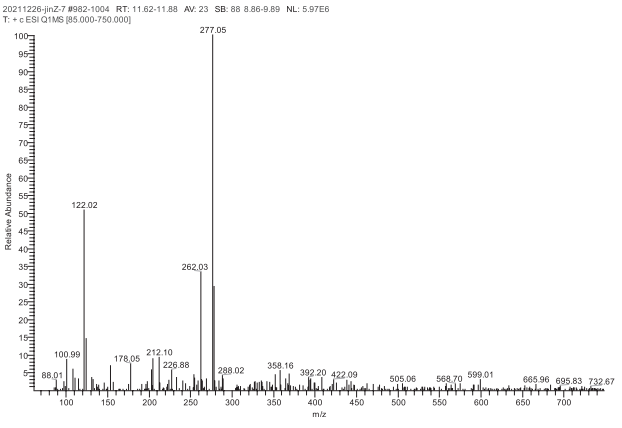


Fig. S4. Continued.

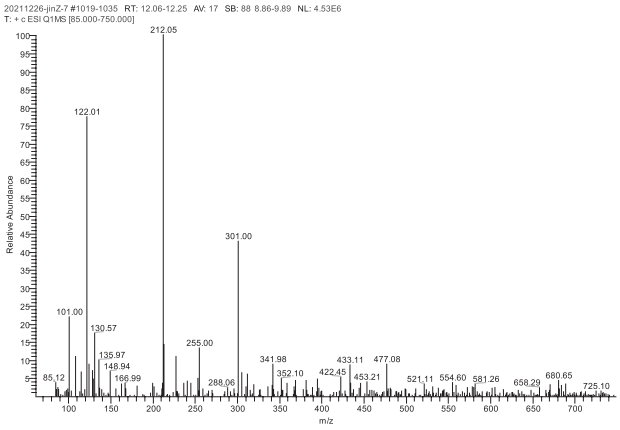


Fig. S4. Continued.