

# Treatment of Antibiotic Residues of Fluoroquinolones (Ofloxacin) in Hospital Wastewater using Peroxone Oxidation Process

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In recent years, the presence of antibiotics in the environment has become a new threat to living organisms. Many advanced treatment technologies have been applied to treat antibiotic residues, especially in advanced oxidation processes. The traditional wastewater treatment process can be replaced by advanced oxidation (AOP), highly reactive free radicals, especially hydroxyl radicals ( $\bullet\text{OH}$ ) generated via Fenton ( $\text{H}_2\text{O}_2/\text{Fe}$ ), peroxone ( $\text{O}_3/\text{H}_2\text{O}_2$ ), electrochemical oxidation, photochemical, serve as the main oxidant. In this study, antibiotic residues of Fluoroquinolones (Ofloxacin) are assessed in hospital wastewater after treatment and evaluated factors affecting peroxone oxidation process. The results show that the treatment efficiency is 72 % at initial OFL concentration of  $20 \mu\text{g}\cdot\text{L}^{-1}$ ,  $\text{O}_3$  ( $5 \text{ mg}\cdot\text{L}^{-1}$ ) flow rate is  $1 \text{ L}\cdot\text{min}^{-1}$ , initial  $\text{H}_2\text{O}_2$  concentration of  $50 \text{ mg}\cdot\text{L}^{-1}$  at pH 7. The OFL removal efficiency by peroxone process decreased when inorganic ions ( $\text{CO}_3^{2-}$ ,  $\text{HCO}_3^-$ ,  $\text{Cl}^-$ ) were added at the concentration of 1mM. . The peroxone process is a promising technique to replace traditional technologies to treat antibiotic residues in wastewater.

## 1. Introduction

Antibiotics can act as ecological factors in the environment, altering the structure of natural microbial communities. Lien et al. (2016) have shown that the presence of antibiotic residues reduces the biodiversity of microorganisms. The effects of antibiotics on ecological functions are alterations in nitrogen metabolism, methanogenesis, reduction of sulfates, changes in the vegetative cycle (Jones et al., 2005).

According to a report by the United States Environmental Protection Agency, many pharmaceutical compounds are now bypassing wastewater treatment stations and entering the aquatic environment through discharge (Jones et al., 2005). The presence of antibiotics in the aquatic environment is of great concern due to the proliferation of antibiotic-resistant bacteria. Zhuang et al. (2020) reported antibiotic residues in hospital wastewater samples, containing mainly fluoroquinolones (79-100 wt%), sulfonamides (86-100 wt%) and macrolides (79-86 wt%). Bu et al. (2013) have shown that some antibiotics are easily degraded, such as penicillin, but some are very stable, difficult to degrade and accumulate high, such as fluoroquinolones (ofloxacin - OFL, ciprofloxacin - CIP), macrolides (tylosin).

The negative effects of antibiotic residues in water sources have been well documented. Anh et al. (2008) reported the low efficiency of biotechnological treatment of fluoroquinolone antibiotics. Another study noted that there was not much difference between the active ingredients of antibiotics in wastewater before and after treatment. This shows that the removal efficiency of these compounds is very low with traditional technology.

The OFL is a very stable antibiotic belonging to the fluoroquinolones group that is commonly detected in wastewater (Guerra et al., 2014) and surface water samples (Bu et al., 2013). The OFL is one of the most commonly used drugs without a prescription, especially in rural areas. The process of bioaccumulation of the antibiotic OFL is increasingly alarming. The problem of removing OFL from wastewater before being discharged into the environment has received great attention in recent years (Lien et al., 2016).

Various techniques have been studied for the removal of antibiotics from water and wastewater, including coagulation (Wang and Wang, 2019), membrane separation (Zhuang et al., 2020), adsorption (Aslvarez-

Torrellas et al., 2016) and biodegradation (Harrabi et al., 2017). Advanced oxidation processes (AOPs) as Fenton ( $\text{H}_2\text{O}_2/\text{Fe}$ ) (Ahmad et al., 2020), peroxone ( $\text{O}_3/\text{H}_2\text{O}_2$ ) (Yao et al., 2014), electrochemical oxidation (Chen et al., 2013) and photochemical (Yaser et al., 2015) were studied to treat antibiotic residues or reduce the inhibitory effect of antibiotics, enhance biodegradability.

In this work, the degradation of OFL by the peroxone process was investigated. The influence of operational conditions and inorganic anions on the mineralization of OFL was also studied.

## 2. Material and methods

### 2.1 Wastewater and chemicals

Wastewater was obtained from equalization tanks at twelve hospital wastewater treatment plants as National Hospital of Odonto-Stomatology, Thong Nhat Hospital, Hung Vuong Hospital, Saigon General Hospital, Gia Dinh Hospital, Cho Ray Hospital, Oncology Hospital, Binh Dan Hospital, Tam Duc Heart Hospital, Hospital for Traumatology and Orthopaedics, Saigon International Obst-Gynec Hospital and Nguyen Tri Phuong Hospital, in Ho Chi Minh city (Vietnam). The wastewater was collected in glass containers and preserved at 4 °C for treatment by advanced oxidation processes. Target antibiotics were purchased from The Institute of Drug Quality Control in Ho Chi Minh city. Analytical-grade standards of OFL (> 98 % purity) were used. HPLC solvents, such as methanol, acetonitrile, ammonium hydroxide solution, acetic acid, formic acid, and other chemicals were analytical grade. The solutions were kept in the dark and stored at 4 °C.

### 2.2 Analysis method

The concentration of OFL was determined by high-performance liquid chromatography equipped with mass spectrometry (LC-MS). An Agilent HPLC system (Model 1200 series) with an Agilent 6130 Series Quadrupole LC-MS System were utilized to monitor the antibiotics extracted from the wastewater using the solid-phase microextraction method as follows. The chromatographic separation was performed on a Zorbax SB-C18 column (150 mm × 4.6 mm) packed with a C18 stationary phase having a particle size of 5 µm. The mobile phase includes solvent A (0.1 % formic acid (v/v) in deionized water) and solvent B (0.1 % formic acid (v/v) and acetonitrile). The flow rate is held at 0.5 mL.min<sup>-1</sup>. The pH was also monitored by a pH meter (InPro4260/120/PT1000, Mettler Toledo, USA) through the multiparameter analyser. Determination of hydroxyl radicals by trap method with dimethyl sulfoxide (DMSO) and liquid chromatography with detector ultraviolet-visible (LC-UV);  $\text{O}_3$  and  $\text{H}_2\text{O}_2$  concentrations were determined by titration method  $\text{Na}_2\text{S}_2\text{O}_3$  and  $\text{KMnO}_4$ .

### 2.3 Experimental design and procedure

Evaluation of the presence of antibiotic residues of OFL in wastewater after treatment of hospitals in Ho Chi Minh city by LC-MS method. The reactions of OFL degradation and mineralization were performed in a 1 L glass reactor, which contained 500 mL OFL aqueous solution (initial concentration was 20 µg.L<sup>-1</sup>) and a certain dosage of  $\text{H}_2\text{O}_2$ . Ozone ( $\text{O}_3$ ) gas was generated using an  $\text{O}_3$  generator with a pure oxygen source and its concentration in the gas phase was monitored with an  $\text{O}_3$  analyser. Ozone (0.5 mg.L<sup>-1</sup>) was continuously pumped into the reaction solution from the bottom of the reactor with a flow rate of 1 L.min<sup>-1</sup> and the residual  $\text{O}_3$  in tail gas was removed by sodium thiosulfate solution. The experiment was performed with simulation wastewater samples to determine the influencing factors: the change of  $\text{O}_3$  and  $\text{H}_2\text{O}_2$  ratio by a concentration of  $\text{H}_2\text{O}_2$ , concentration initial of OFL, pH, inorganic ions ( $\text{CO}_3^{2-}$ ,  $\text{HCO}_3^-$ ,  $\text{Cl}^-$ ).

## 3. Results and discussion

### 3.1 Evaluation of the presence of antibiotic residues of OFL in the wastewater of hospitals.

Wastewater samples before and after treatment were collected at wastewater treatment systems of 12 hospitals in Ho Chi Minh city (Vietnam). These samples were taken and preserved according to the requirements stated in Section 2.1 and analysed for the concentration of OFL present by the LC-MS method as described in Section 2.2. Table 1 shows the presence of OFL in samples before and after the hospital wastewater treatment systems. This antibiotic is found in hospitals such as Saigon General Hospital, Cho Ray Hospital, Oncology Hospital, Binh Dan Hospital, Nguyen Tri Phuong Hospital and Tam Duc Heart Hospital. The highest is Saigon General Hospital and Nguyen Tri Phuong Hospital with relatively high influent and effluent concentrations: influent  $48.85 \pm 2.76$  µg.L<sup>-1</sup> and  $39.89 \pm 1.76$  µg.L<sup>-1</sup>; effluent  $19.95 \pm 1.22$  µg.L<sup>-1</sup> and  $19.71 \pm 1.47$  µg.L<sup>-1</sup>. This can be explained that because these are upper-level general hospitals, receiving a large number of patients in Ho Chi Minh city and surrounding areas. The OFL is a strong antibiotic with many side effects, so most hospitals do not give priority to using this antibiotic. In all 12 hospitals, only 6 hospitals use antibiotic ofloxacin in a limited way. This is an antibiotic that is difficult to degrade, so it can accumulate in high concentrations in water (Lien et al., 2016).

Table 1 also shows that the efficiency of wastewater treatment containing OFL antibiotic residues by traditional biotechnology is only from 37.1 % to 59.5 %.

Table 1: The presence of OFL before and after treatment from wastewater of hospitals

Hospital	OFL influent ( $\mu\text{g.L}^{-1}$ )	OFL effluent ( $\mu\text{g.L}^{-1}$ )	Efficiency (%)
Saigon General Hospital	$48.85 \pm 2.76$	$19.95 \pm 1.22$	59.2
Cho Ray Hospital	$24.21 \pm 1.05$	$15.32 \pm 1.65$	37.1
Oncology Hospital	$22.12 \pm 1.13$	$12.23 \pm 0.95$	44.7
Binh Dan Hospital	$13.78 \pm 0.45$	$5.58 \pm 0.31$	59.5
Tam Duc Heart Hospital	$0.55 \pm 0.21$	-	-
Nguyen Tri Phuong Hospital	$39.89 \pm 1.76$	$19.71 \pm 1.47$	50.6

"-": No data

### 3.2 Degradation of OFL by peroxone process

#### 3.2.1 Effect of pH on OFL removal in peroxone processes

The effect of pH on OFL removal at pH values (3, 5, 7, 9, 11) shows in Figure 1. The OFL treatment efficiency of  $\text{O}_3$  is higher than  $\text{H}_2\text{O}_2$  and is highest when combining  $\text{O}_3$  and  $\text{H}_2\text{O}_2$ . When the pH increased from 3 to 9, the treatment efficiency increased for all 3 experiments. At the pH = 11, the treatment efficiency decreased. The highest treatment efficiency at pH = 7 is 72 %. At pH = 7, a portion of the  $\text{O}_3$  in the solution will decompose to produce free radicals such as  $\cdot\text{OH}$ , which has a high redox potential ( $E_0 = +2.80 \text{ V}$ ). In peroxone process, the decomposition of  $\text{O}_3$  in the solution would be improved by  $\text{H}_2\text{O}_2$ , resulting in the formation of  $\cdot\text{OH}$  (Lin et al., 2009). After 60 min, the treatment efficiency reached over 55 % at the pH values. The combination of  $\text{O}_3$  and  $\text{H}_2\text{O}_2$  shows significant improvement in treatment efficiency compared to  $\text{O}_3$  and  $\text{H}_2\text{O}_2$  alone. After 30 min, the OFL treatment efficiency reached over 45 % for  $\text{O}_3$  and 35 % for  $\text{H}_2\text{O}_2$ , but when  $\text{O}_3$  and  $\text{H}_2\text{O}_2$  were combined in the peroxone process, the treatment efficiency reached over 50 % and increased to 72 % after 60 min. Chen and Wang (2021) showed OFL removal efficiency (pH = 7) reached 35 % after 30 min and 45 % after 60 min.

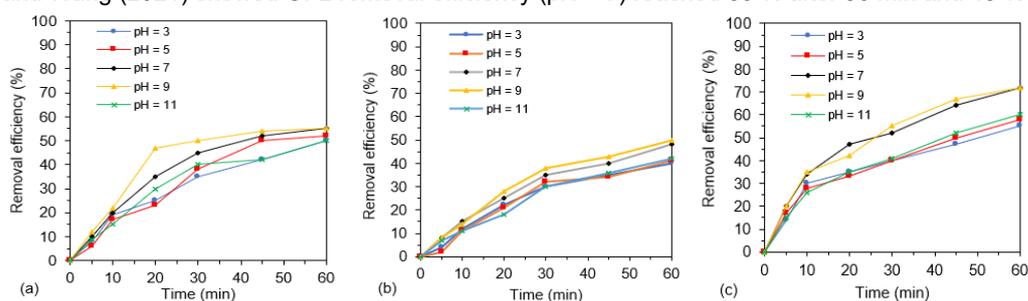


Figure 1: Effect of pH on OFL removal in peroxone processes: (a) Single  $\text{O}_3$ , (b) Single  $\text{H}_2\text{O}_2$  and (c)  $\text{O}_3/\text{H}_2\text{O}_2$  ( $\text{OFL}_{\text{initial}} = 20 \mu\text{g.L}^{-1}$ ;  $\text{O}_3 = 5 \text{ mg.L}^{-1}$ ;  $\text{O}_3$  gas flow rate of  $1 \text{ L.min}^{-1}$ ;  $\text{H}_2\text{O}_2_{\text{initial}} = 50 \text{ mg.L}^{-1}$ ).

The peroxone process forms  $\cdot\text{OH}$  in the interaction between  $\text{O}_3$  and  $\text{H}_2\text{O}_2$  according to the equations from Eq(1) to Eq(9).





As shown in Figure 2, the decomposition of  $\text{O}_3$  were motivated by the addition of  $\text{H}_2\text{O}_2$ . The concentration of  $\text{O}_3$  in the solution was relatively low in the initial 10 min of the reaction, no enough oxidants were available for OFL degradation. Although the formed  $\bullet\text{OH}$  might be more powerful in the oxidation of OFL, the lower yield of  $\bullet\text{OH}$  at the initial stage of the reaction, leading to less  $\bullet\text{OH}$  for OFL degradation. After 20 min, the decomposition of  $\text{O}_3$  and  $\text{H}_2\text{O}_2$  into  $\bullet\text{OH}$  increased rapidly when the concentration of  $\text{O}_3$  and  $\text{H}_2\text{O}_2$  in the solution increased, reaching the maximum value after 30 min. The decomposition of  $\text{H}_2\text{O}_2$  takes place more rapidly in the presence of  $\text{O}_3$  than in the case of only  $\text{O}_2$ .

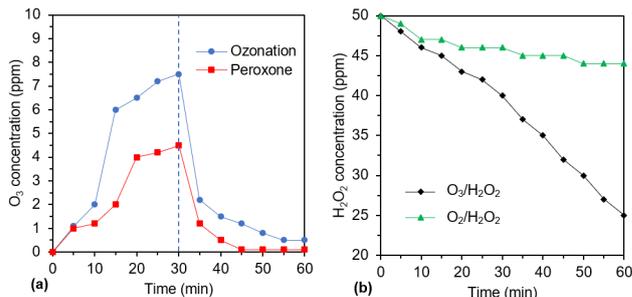


Figure 2: The increase in  $\text{O}_3$  concentration and the decomposition of  $\text{O}_3$  (a); the decomposition of  $\text{H}_2\text{O}_2$  in solutions (b) ( $\text{O}_3 = 5 \text{ mg}\cdot\text{L}^{-1}$ ;  $\text{O}_3$  or  $\text{O}_2$  gas flow rate of  $1 \text{ L}\cdot\text{min}^{-1}$ ; initial pH of 7;  $\text{H}_2\text{O}_2_{\text{initial}} = 50 \text{ mg}\cdot\text{L}^{-1}$ ).

### 3.2.2 Effect of $\text{H}_2\text{O}_2$ on OFL removal in peroxone processes

The degradation of OFL by the  $\text{O}_3/\text{H}_2\text{O}_2$  process at different concentrations of  $\text{H}_2\text{O}_2$  ( $0\text{--}100 \text{ mg}\cdot\text{L}^{-1}$ ) is illustrated in Figure 3. The OFL removal was improved in the presence of  $\text{H}_2\text{O}_2$  and the high treatment efficiency of OFL (72 %) was achieved at the application of  $50 \text{ mg}\cdot\text{L}^{-1}$   $\text{H}_2\text{O}_2$ , no more than 50 % OFL removal was observed in  $\text{O}_3$  alone process. The removal efficiency increased with increasing  $\text{H}_2\text{O}_2$  concentration from 0 to  $50 \text{ mg}\cdot\text{L}^{-1}$ , while there was no further enhancement of OFL removal with further rising the concentration of  $\text{H}_2\text{O}_2$ . More  $\text{H}_2\text{O}_2$  would accelerate  $\text{O}_3$  decomposition, yielding more  $\bullet\text{OH}$ , which could promote the oxidation of the intermediate formed during the reaction (Staehelin and Hoigne, 1982). Since excess  $\text{H}_2\text{O}_2$  might act as a  $\bullet\text{OH}$  scavenger, which would scavenge the formed  $\bullet\text{OH}$  leading to the inhibition of OFL removal (Chen et al., 2015). Chen and Wang (2021) reported that increasing the  $\text{H}_2\text{O}_2$  concentration from  $0\text{--}40 \text{ mg}\cdot\text{L}^{-1}$  increased the OFL removal efficiency, but further increasing the  $\text{H}_2\text{O}_2$  concentration  $40\text{--}100 \text{ mg}\cdot\text{L}^{-1}$ , the treatment efficiency did not change significantly. The OFL removal efficiency reaches 55 % after 60 min

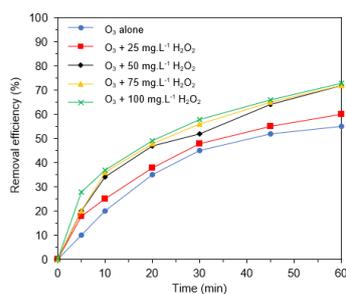


Figure 3: Effect of  $\text{H}_2\text{O}_2$  on OFL removal in peroxone processes ( $\text{OFL}_{\text{initial}} = 20 \mu\text{g}\cdot\text{L}^{-1}$ ;  $\text{O}_3 = 5 \text{ mg}\cdot\text{L}^{-1}$ ;  $\text{O}_3$  gas flow rate of  $1 \text{ L}\cdot\text{min}^{-1}$ ; initial pH of 7)

### 3.2.3 Effect of $\text{O}_3$ on OFL removal in peroxone processes

The effect of  $\text{O}_3$  concentration on OFL removal was also investigated in Figure 6. The removal of OFL was positively correlated with  $\text{O}_3$  concentration. In the absence of  $\text{O}_3$ , no OFL removal could be observed, suggesting that OFL or its intermediates were hardly oxidized by  $\text{H}_2\text{O}_2$ . In peroxone process, increasing  $\text{O}_3$  concentration, more  $\text{O}_3$  and free radicals in solution were available, resulting in the improvement of OFL removal. When  $\text{O}_3$  dosage increased from 5 to  $10 \text{ mg}\cdot\text{L}^{-1}$ , the enhancement of OFL removal was not so obvious as that from 0 to

5 mg.L<sup>-1</sup>. Since •OH could also react with O<sub>3</sub>, indicating there may be an optimum dose ratio of H<sub>2</sub>O<sub>2</sub> and O<sub>3</sub> which was also in accordance with the results of the effect of different H<sub>2</sub>O<sub>2</sub> concentrations on OFL removal. As the O<sub>3</sub> concentration continued to increase, the OFL reduction efficiency increased but not significantly (Chen and Wang, 2021).

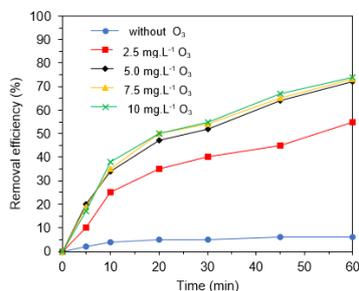


Figure 4: Effect of O<sub>3</sub> on OFL removal in peroxone processes (OFL<sub>initial</sub> = 20 µg.L<sup>-1</sup>; initial pH of 7; O<sub>3</sub> gas flow rate of 1 L.min<sup>-1</sup>; H<sub>2</sub>O<sub>2</sub> initial = 50 mg.L<sup>-1</sup>)

### 3.2.4. Effect of OFL concentration initial on OFL removal in peroxone processes

Figure 5 shows the effect of the OFL concentration initial on the removal of OFL. Since increasing the concentration of OFL initial from 20 to 30 mg.L<sup>-1</sup>, more oxidant was needed to oxidize OFL and its intermediates, the removal efficiency of OFL decrease. At OFL concentration (10 mg.L<sup>-1</sup>), OFL removal were highest (74 %).

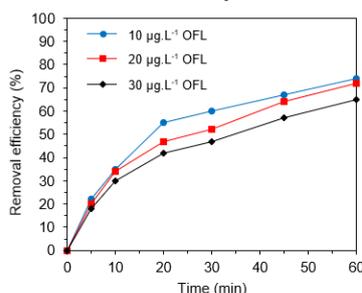


Figure 5: Effect of OFL concentration initial on OFL removal in peroxone processes (O<sub>3</sub> = 5 mg.L<sup>-1</sup>; O<sub>3</sub> gas flow rate of 1 L.min<sup>-1</sup>; initial pH of 7; H<sub>2</sub>O<sub>2</sub> initial = 50 mg.L<sup>-1</sup>)

### 3.2.5. Influences of inorganic ions HCO<sub>3</sub><sup>-</sup>, CO<sub>3</sub><sup>2-</sup>, Cl<sup>-</sup> on OFL removal in peroxone processes

Anions such as HCO<sub>3</sub><sup>-</sup>, CO<sub>3</sub><sup>2-</sup> and Cl<sup>-</sup> were commonly present in the natural water environment and may have some effect on the removal of organics in ozonation process. The influence of HCO<sub>3</sub><sup>-</sup>, CO<sub>3</sub><sup>2-</sup> and Cl<sup>-</sup> (1 mM) on the removal of OFL by peroxone process was shown in Figure 7. In the presence of HCO<sub>3</sub><sup>-</sup>, CO<sub>3</sub><sup>2-</sup> and Cl<sup>-</sup> ions (1 mM), the removal of OFL was inhibited in peroxone process. Since the HCO<sub>3</sub><sup>-</sup>, CO<sub>3</sub><sup>2-</sup> and Cl<sup>-</sup> might consume O<sub>3</sub> under the conditions in this work, the enhancement or inhibition of OFL removal mostly resulted from the effect of the anions on the formation and quenching of •OH during O<sub>3</sub>/H<sub>2</sub>O<sub>2</sub> process. Chen and Wang (2021) showed that HCO<sub>3</sub><sup>-</sup> ion promotes OFL reduction while Cl<sup>-</sup>, PO<sub>4</sub><sup>3-</sup> and NO<sub>3</sub><sup>-</sup> ions inhibit •OH formation in peroxone.

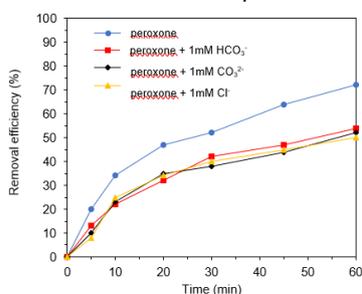


Figure 6: Influences of inorganic ions on OFL removal by peroxone process: (a) HCO<sub>3</sub><sup>-</sup>; (b) CO<sub>3</sub><sup>2-</sup> and (c) Cl<sup>-</sup> (OFL<sub>initial</sub> = 20 µg.L<sup>-1</sup>; O<sub>3</sub> = 5 mg.L<sup>-1</sup>; O<sub>3</sub> gas flow rate of 1 L.min<sup>-1</sup>; initial pH of 7; H<sub>2</sub>O<sub>2</sub> initial = 50 mg.L<sup>-1</sup>).

#### 4. Conclusion

The study evaluated the residual status of OFL in 12 hospitals in Ho Chi Minh city, Vietnam. The amount of residual OFL in hospital wastewater after treatment is still high, which can be dispersed and accumulated in surface water. The peroxone process in the study showed an OFL treatment efficiency of up to 72%, higher than that of biotechnology (only less than 60%). Factors affecting the peroxone process such as initial OFL concentration,  $O_3$  and  $H_2O_2$  concentration, pH and inorganic ions were also evaluated. The results show efficiency to decompose OFL rapidly both in single  $O_3$  process and  $O_3/H_2O_2$  process. When  $O_3$  is combined with  $H_2O_2$ , OFL mineralization is greatly improved due to the improved formation of  $\bullet OH$ , resulting in a high removal of OFL. The pH value decreased rapidly during the reaction, different initial pH had little effect on OFL removal. Different anions can have different effects on OFL removal, related to their effects on  $\bullet OH$  radicals.

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