Enhanced biodegradation of ciprofloxacin by enrich nitrifying sludge: assessment of removal pathways and microbial responses

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Abstract

Antibiotics are mostly collected by sewage systems, but not completely removed within wastewater treatment plants. Their release to aquatic environment poses great threat to public health. This study evaluated the removal of a widely used fluoroquinolone antibiotic ciprofloxacin in enriched nitrifying culture through a series of experiments by controlling ammonium concentrations and inhibiting functional microorganisms. The removal efficiency of ciprofloxacin at an initial concentration of 50 μg/L reached 81.86 ± 3.21% in the presence of ammonium, while only 22.83 ± 8.22% of ciprofloxacin was removed in its absence. The positive linear correlation was found between the ammonia oxidation rate (AOR) and ciprofloxacin biodegradation rate. These jointly confirmed the importance of the AOB-induced cometabolism in ciprofloxacin biodegradation with adsorption and metabolic degradation pathways playing minor roles. The continuous exposure of AOB to ciprofloxacin led to decreases of ammonia monooxygenase (AMO) activities and AOR. The antibacterial effects of ciprofloxacin and its biodegradation products were further evaluated and the results revealed that biodegradation products of ciprofloxacin exhibited less toxicity compared to the parent compound, implying the potential application of cometabolism in alleviation of antimicrobial activity. The findings provided new insights into the AOB-induced cometabolic biodegradation of fluoroquinolone antibiotics.

Key words: ammonia monooxygenase, ammonia oxidation rate, antibacterial effects, ciprofloxacin, cometabolic biodegradation, nitrifying sludge

Highlights

- Cometabolic biodegradation was the major removal pathway of ciprofloxacin.
- Contributions from metabolisms by AOB and heterotrophs were insignificant.
- AOR, AMO activities and amoA gene abundance decreased during exposure.
- Cometabolic biodegradation products exhibited less toxicity than ciprofloxacin.
INTRODUCTION

The occurrence of emerging micropollutants in the aquatic environment has received widespread attention due to their cytotoxicity or ecotoxicity in recent years (Bielen et al. 2017; Aemig et al. 2021). Among the emerging micropollutants, antibiotics are commonly used to treat human infections and promote animal growth, contributing to their concentrations at single ng L$^{-1}$ to a few hundreds of μg L$^{-1}$ in the aquatic environment (Depeursinge et al. 2010; Luo et al. 2014; Oberoi et al. 2019). The presence of antibiotics in the environment might lead to changes in microbial community structure and microbial diversity, thereby disturbing the balance of the ecosystem (Hernando et al. 2006; Zhao et al. 2021). The other risk issue is the emergence and spread of antibiotic resistance genes (ARGs) regarding the long-term presence of antibiotics in the environment (Depeursinge et al. 2010; Van Doorslaer et al. 2014). These ARGs promote the appearance of antibiotic resistant bacteria (ARBs), which pose a threat to animal and human health (Aydin et al. 2016; Chaturvedi et al. 2021).

Incomplete removal of antibiotics in the conventional wastewater treatment processes resulted in antibiotic residues and their degradation products entering the environment. Degradation products might be more toxic than the parent compound (Xu et al. 2016). It was reported that removal of antibiotics could be enhanced under nitrifying conditions (Shi et al. 2011; Dorival-García et al. 2013; Dawas-Massalha et al. 2014; Men et al. 2017; Ooi et al. 2018; Ramírez Muñoz et al. 2020; Wang et al. 2020). A positive linear correlation was found between the antibiotic biodegradation rate and the ammonium oxidation rate (AOR) when sufficient ammonium was provided (Tran et al. 2009; Fernandez-Fontaina et al. 2014; Wang et al. 2019a, 2019b; Zhou et al. 2019). Ammonia-oxidizing bacteria (AOB) in nitrifying activated sludge could degrade a broad spectrum of aromatic compounds as a result of cometabolism by non-specific ammonia monooxygenase (AMO) (Keener & Arp 1994; Arp et al. 2001; Skotnicka-Pitak et al. 2009). The activity of nitrifying communities could be assessed by analysis of the functional amoA gene (i.e., AMO subunit A) from the molecular level (Chen 2017).

Ciprofloxacin is the most widely used fluoroquinolone antibiotics due to its activity against a wide range of Gram-negative and Gram-positive bacteria (Davis et al. 1996; Picó & Andreu 2007). Zhou et al. (2021) found that pure cultures of AOB or ammonia oxidizing archaea (AOA) instead of the complete ammonia oxidizer (comammox) could significantly biotransformed ciprofloxacin and norfloxacin via cometabolism. Although nitrifying bacteria were already known to exhibit remarkable performance in the removal of ciprofloxacin (Dorival-García et al. 2013; Wang et al. 2017), little information was yet available on the interactions between the AOB activity and fluoroquinolone (e.g., ciprofloxacin) along with its degradation products so far.

The main objective of this work is to investigate the biodegradation of ciprofloxacin in the enriched nitrifying sludge and elucidate the interaction between the target compound and responsible microorganisms. Removal of ciprofloxacin was studied under controlled conditions to assess the contributions from metabolism, cometabolism and adsorption. The roles of responsible microorganisms in the enriched nitrifying sludge were also evaluated during removal of ciprofloxacin.
effect of ciprofloxacin exposure was assessed on AOB activity including ammonia oxidation activity and key enzyme activity. The antibacterial activities of ciprofloxacin biodegradation products were also explored through a series of biotoxicity tests.

**MATERIALS AND METHODS**

**Chemicals**

Ciprofloxacin (>98%), ciprofloxacin-d8 and allylthiourea (ATU, 98%) were purchased from Sigma-Aldrich, China. Methanol, acetonitrile, formic acid and all the other organic solvents (HPLC grade) were purchased from ThermoFisher, USA. For individual standard stock solution, ciprofloxacin was prepared in 0.2 M hydrochloric acid (HCl, Analytical grade) at 100 mg L⁻¹ and stored at −20 °C. Working standards were prepared through dilution of the standard stock solution with 0.2 M HCl to establish the standard curve. Ciprofloxacin feeding solution for batch experiments was prepared in 5% HCl at 100 mg L⁻¹. Ciprofloxacin-d8 standard solution was prepared in methanol at 100 mg L⁻¹.

**Enrichment of nitrifying sludge**

Activated sludge from a domestic wastewater treatment plant in Wuhan, China was used as the inoculum to enrich the nitrifying sludge in a sequencing batch reactor (SBR) at a working volume of 4 L (see Fig. S1 in Supporting Information). The operating cycle of the SBR was 6 h, consisting of 260 min aerobic feeding, 30 min aerobic reacting, 1 min wasting, 60 min settling and 9 min decanting. During aerobic feeding, 1 L synthetic wastewater was fed into the reactor following the composition as described in our previous work (Xu et al. 2017a). Hydraulic retention time (HRT) was maintained at 24 h. Dissolved oxygen (DO) was controlled at 2.5–3.0 mg L⁻¹ through the programmed logic controller (PLC) and pH was adjusted between 7.5 and 8 by adding sodium bicarbonate or HCl. The SBR was operated in steady state with more than 98% conversion of NH₄⁺ to NO₃⁻, prior to further degradation experiments. The detailed information on SBR operation and performance was presented as Text S1 and Fig. S2 and S3.

**Ciprofloxacin degradation experiments**

Ciprofloxacin degradation experiments were conducted in 2-L beakers with working volumes of 1.5 L, wrapped in aluminum foil to avoid possible photodegradation. Mixed liquor volatile suspended solids (MLVSS) concentration was maintained at approximately 1 g L⁻¹ for the degradation experiments. The DO concentration and pH were controlled in the range of 3.0–3.5 mg L⁻¹ and 7.5–8, respectively. The initial concentration of ciprofloxacin was 50 μg L⁻¹. Experiment 1 was performed to evaluate the biodegradation of ciprofloxacin in the constant presence of 50 mg L⁻¹ of ammonium, which was realized by refilling a mixture of ammonium bicarbonate and sodium bicarbonate as pH adjustment simultaneously. Experiment 2 was performed to assess ciprofloxacin biodegradation in the absence of ammonium during the entire period. In Experiment 3, 50 mg L⁻¹ of ammonium and 50 mg L⁻¹ ATU were added initially to study the contribution of heterotrophic bacteria to the biodegradation of ciprofloxacin. ATU was reported to be a potent and selective inhibitor of ammonia oxidation by chelating copper at the AMO active site (Ginestet et al. 1998; Ali et al. 2013). Experiment 4 was used as a control to evaluate the contribution of abiotic degradation, where enriched nitrifying sludge was autoclaved at 121 °C, 103 kPa for 30 min to ensure complete inactivation of microbial activity. All batch degradation experiments were carried out with a magnetic stirrer at 250 rpm. The detailed experimental conditions were summarized in Table S1 in SI. Samples were taken periodically for further analysis of ciprofloxacin and microbial activity.

**Analytical methods**

The concentration of ciprofloxacin was detected using ultra performance liquid chromatograph coupled with quadrupole mass spectrometer equipped with an electrospray ion source (ACQUITY UPLC H-class Xevo TQ MS, Waters, USA). Chromatographic separation was performed using an ACQUITY UPLC BEH C18 column (2.1 × 50 mm, 1.7 μm) at 40 °C. The injection volume was 2 μL. The mobile phases were (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile with a total flow rate of 0.2 mL min⁻¹. The gradient elution program was: 85% A and 15% B for 4 min. The total run time was 4 min. The mass spectrometry analyses were operated using an electrospray (ESI) source in positive mode with the following parameters: 3 kV capillary voltage, 30 V cone voltage, 350 °C desolvation temperature, and 650 L h⁻¹ desolvation gas (nitrogen >99.999%) flow. Data acquisition was performed using MassLynx V 4.1 software with the Quanlynx program (Waters, USA).
The concentrations of NH$_4^+$-N, NO$_2^-$-N and NO$_3^-$-N were measured according to Nessler’s reagent colorimetric method, N-(1-naphthyl)-ethylenediamine dihydrochloride colorimetric method and ultraviolet spectrophotometry, respectively, after filtering the samples with 0.22 μm polyethersulfone (PES) filters. The mixed liquid suspended solid (MLSS) concentration and MLVSS concentration were measured in triplicate following the standard method (Clesceri et al. 1999).

**AMO enzyme activity test**

8 mL sludge samples were centrifuged at 4,000 rpm for 15 min at 4 °C. Afterwards, the pellet was washed 3 times with 0.01 M phosphate buffer (pH = 7.4) and resuspended with 8 mL of 0.01 M phosphate buffer. The suspensions were fragmented for 5 min under ice bath conditions by an ultrasonic cell crusher (SCIENTZ, China) at intervals and working time of 10 and 5 s, respectively. The cell fragments were centrifuged at 11,000 rpm at 4 °C for 15 min. The supernatant, i.e., crude enzyme extract, was stored at 4 °C for enzyme activity analysis within 24 h.

AMO enzyme activity assays were conducted in triplicate in 50 mL centrifuge tubes where 2 mL of crude extract and 18 mL of 0.01 M phosphate buffer containing 2 mM NH$_4$Cl were mixed well. 2 mL mixture was initially taken for NH$_4^+$-N analysis, and then cultivated in a shaking incubator at 30 °C for 30 min with the lid open. The NH$_4^+$-N in the mixture was analyzed again at the end of the test. The protein content of each sample was determined according to the modified Lowry method described by (Hülsen et al. 2018). The AMO enzyme activity was determined based on the decrease of NH$_4^+$-N in the mixture as mg N g protein$^{-1}$ min$^{-1}$.

**Antibacterial activity measurement**

*Escherichia coli* K12 (*E. coli* K12, Preservation No. CCTCC AB 2014342; CCTCC, China) was selected to further investigate the antibacterial activity of ciprofloxacin and its degradation products according to the reported methods (Liang et al. 2013; Zheng et al. 2020). *E. coli* K12 was incubated overnight in Luria Bertani (LB) medium. For different tests, 2 mL *E. coli* K12 in the exponential phase were inoculated into the following mediums in triplicate each: (a) 148 mL LB medium to supply as the negative control; (b) 148 mL LB medium spiked with 50 μg L$^{-1}$ ciprofloxacin; (c) 148 mL LB medium prepared in the mixed liquor withdrawn at the end of Experiment 1, which was prefiltered with a GF/C glass fiber filter (Whatman, UK). The average initial optical density (OD) value determined for these tests was 0.063. All tests were shaken in the incubator at 120 rpm for 20 h and 4 mL samples were taken every 2 h to measure the OD at 600 nm.

**Statistical analysis**

All batch experiments were performed in duplicate and the mean ± standard deviation values were reported. One-way analysis of variance (ANOVA) was used to evaluate significant differences in the removal efficiency of ciprofloxacin under different controlled conditions, and *p*-value less than 0.05 was regarded as statistically significant.

**RESULTS**

**Removal of ciprofloxacin in the presence of ATU**

The concentrations of ciprofloxacin under all experimental conditions were listed in Table S2. Ciprofloxacin concentration decreased rapidly during the first 48 h and then remained nearly constant until the end of the experiment, achieving an average removal efficiency of 15.90 ± 0.26% in the abiotic control (see Figure 1). The contribution of photolysis to ciprofloxacin removal was negligible owing to the wrapped aluminum foil. Losses due to volatilization and hydrolysis were also negligible, based on the previously reported Henry’s law constant (5.09 × 10$^{-19}$ atm·m$^3$ mol$^{-1}$) of ciprofloxacin and its inherent insolubility in water (<1 mg L$^{-1}$) (Wang et al. 2017; Rao et al. 2021). Therefore, the obtained removal efficiency of 15.90 ± 0.26% was mainly attributed to the adsorption of inactivated nitrifying biomass.

In the presence of 50 mg L$^{-1}$ ATU, the constant ammonium concentration profile indicated a complete inhibition of ammonia oxidation activity (Fig. S4), excluding the contribution of nitrifying bacteria to the removal of ciprofloxacin. As demonstrated in Figure 1, the average removal efficiency of ciprofloxacin was 21.11 ± 3.60% with ATU added. During the initial 48 h, a decreasing trend was observed with the removal efficiency of 17.80 ± 3.80%, which was similar to the situation in the abiotic control. The rapid reduction in ciprofloxacin concentration in both experiments indicated the major role of adsorption at the beginning of experiments. It also suggested that heterotrophic bacteria probably played a minor role in removal of ciprofloxacin, with contribution of heterotrophic biodegradation less than 5.21%. As no organic substrates were provided during the experiment, metabolism induced by heterotrophs should be the major biodegradation mechanism.
Removal of ciprofloxacin in the absence of ammonium

The average removal efficiency of ciprofloxacin was 22.83 ± 8.22% in the absence of ammonium as indicated in Figure 1, which was higher than the values in the abiotic control experiment (15.90 ± 0.26%) and in the ATU-added experiment (21.11 ± 3.60%). Significant difference was obtained for the ciprofloxacin removal between the experiment in the absence of ammonium and abiotic control experiment (p < 0.05). Previous studies reported that pharmaceuticals (e.g., atenolol, cephalaxin, sulfadiazine) could also be degraded by enriched nitrifying sludge with no ammonium provided (Xu et al. 2017a; Wang et al. 2019a, 2019b). Given the absence of primary substrate and energy source, the process of ammonia oxidation was not favored, thus the contribution of AOB-induced cometabolism to ciprofloxacin removal is negligible.

Comparing removal efficiencies in absence of ammonium and in the presence of ATU, it was proposed that insignificant contribution was conducted by AOB-induced metabolic biodegradation to ciprofloxacin removal.

Removal of ciprofloxacin in the presence of ammonium

Ammonium concentrations were controlled at nearly constant 50 mg N L⁻¹ in the experiments with the presence of ammonium (see Figure 2). As shown in Figure 1, ciprofloxacin continuously reduced from an initial concentration of 38.76 ± 2.42 μg L⁻¹ to 6.99 ± 0.81 μg L⁻¹, yielding an average removal efficiency of 81.86 ± 3.21%. The decreasing trend followed the pseudo first order degradation kinetics (Fig. S5). In the first 48 h of the experiment, ciprofloxacin decreased rapidly.

Figure 1 | Removal of ciprofloxacin in the controlled experiments. Y axis indicates the concentration of ciprofloxacin (C) normalized to its initial concentration (C0).

Figure 2 | Changes in NH₄⁺-N, NO₂⁻-N, and NO₃⁻-N concentrations in the experiments with the presence of ammonium.
with the average degradation rate of 0.25 μg gVSSh⁻¹. The average degradation rate decreased to 0.20 μg gVSSh⁻¹ in the subsequent 48–96 h while the corresponding rate was only 0.16 μg gVSSh⁻¹ in the remaining 48 h of the experiment. The highest removal efficiency and the relative high biodegradation rate obtained in the presence of ammonium would be ascribed to the cometabolic biodegradation induced by AOB, as the sufficient ammonium was provided during the entire experiment. This notion was also confirmed in previous studies on biodegradation of norfloxacin, sulfadimidine, metoprolol, carbamazepine, bezafibrate and griseofulvin under nitrification (Suarez et al. 2010; Park et al. 2017). As shown in Figure 3(a), changes in AOR were also monitored with an increasing trend from 23.89 ± 7.75 mg NH₄-N gVSS h⁻¹ to 33.02 ± 2.44 mg NH₄-N gVSS h⁻¹ during the first 24 h. The subsequent 120 h witnessed the gradual decrease in AOR from 33.02 ± 2.44 mg NH₄-N gVSS h⁻¹ to 15.49 ± 2.08 mg NH₄-N gVSS h⁻¹. Simultaneously, the ciprofloxacin biodegradation rate decreased from 0.27 μg gVSSh⁻¹ to 0.14 μg gVSSh⁻¹. Figure 3(b) demonstrated a positive linear correlation between AOR and ciprofloxacin biodegradation rate, which also indicated the cometabolic biodegradation in the presence of ammonium.

The changes in AMO activities during cometabolic biodegradation of ciprofloxacin was plotted in Figure 4. The AMO activity decreased from 0.58 ± 0.06 mg N g protein⁻¹ min⁻¹ to 0.23 ± 0.01 mg N g protein⁻¹ min⁻¹ in response to the presence of ciprofloxacin during the experimental period. This change in AMO activity was consistent with the trend in AOR, indicating that ciprofloxacin had an adverse effect on the activity of nitrifying bacteria. Decreasing AMO activities was

Figure 3 | (a) Changes in the ammonium oxidation rate in the experiment in the presence of ammonium and (b) Relationship curve between the ammonium oxidation rate and the ciprofloxacin biodegradation rate.

Figure 4 | Changes in ammonia monooxygenase activity in the experiment in the presence of ammonium.
also reported to be caused by other antibiotics (e.g., cephalexin, ampicillin, sulfadoxine, tetracycline, etc.) (Huang et al. 2016; Yu et al. 2019). Ciprofloxacin might cause bacterial death by inhibiting DNA synthesis and replication, thus decreasing the production of AMO (Riaz et al. 2018).

**Antibacterial activity of ciprofloxacin biodegradation products**

In this study, *E. coli* K12 was adopted as the model microorganism to assess the antibacterial effect of biodegradation products of ciprofloxacin. Figure 5 depicted the growth curves of *E. coli* K12 in different mediums. It was obvious that *E. coli* K12 grew best as the normal curve in the control group. However, the growth curves were inhibited in the mediums with either ciprofloxacin or its biodegradation products. After exposure of ciprofloxacin for 6 h, the growth curve of *E. coli* K12 showed a decreasing trend, implying the strong biotoxicity of ciprofloxacin. In the medium with addition of biodegradation products formed under cometabolic conditions, *E. coli* K12 proliferated steadily and rapidly notwithstanding the slower growth rate than the control. Compared with the parent compound ciprofloxacin, cometabolic biodegradation products exhibited lower biotoxicity to *E. coli* K12. This might suggest that cometabolism would play an important role in eliminating antibacterial activities of ciprofloxacin.

**DISCUSSION**

**Contributions of different pathways to ciprofloxacin removal**

The contradictory notions have been reported on the contribution of sorption to removal of antibiotics by activated sludge. 50–91% of fluoroquinolone antibiotics (e.g., ciprofloxacin, enrofloxacin, ofloxacin, norfloxacin and lomefloxacin) were removed by adsorption onto activated sludge, which was related to their respective chemical structures and low octanol-water partition coefficients $\log K_{OW} (-1.13$ to 0.27) at pH 7.4–7.5 at 25 °C (Wang et al. 2017; Hu et al. 2018; Rao et al. 2021). However, the insignificant absorption capacity of nitrifying bacteria for micropollutants (e.g., ibuprofen, naproxen, diclofenac, trimethoprim, erythromycin, roxithromycin and fluoxetine) has been observed in previous studies (Fernandez-Fontaina et al. 2012; Deng et al. 2016). Dawas-Massalha et al. (2014) also reported that the removal of pharmaceuticals (i.e., ibuprofen, ketoprofen, carbamazepine, dexamethasone and iopromide) by adsorption was negligible because of high biological activity and low adsorption capacity of nitrifying bacteria. Therefore, it was speculated that adsorption of ciprofloxacin in this work was likely to be mainly contributed by heterotrophic bacteria instead of AOB, requiring further validation.

Apart from the adsorption, AOB-induced metabolic biodegradation and heterotrophic metabolic biodegradation might contributed 6.93% of ciprofloxacin removal, rendering insignificant contribution (1.72%) from AOB-induced metabolic biodegradation. This could probably be ascribed to the toxicity of ciprofloxacin on AOB. AOB could utilize non-toxic organic

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**Figure 5** Growth curves of *E. coli* K12 in different mediums (control group: LB medium; ciprofloxacin group: LB medium containing 50 µg L$^{-1}$ ciprofloxacin; ciprofloxacin and degradation products group: LB medium containing the final sample at the end of Experiment 1). OD600 indicates the cell density of *E. coli* K12 in liquid medium.
micropollutants as potential carbon and nitrogen sources for their own growth, facilitating the metabolic biodegradation (Tran et al. 2013; Harb et al. 2016). For example, Müller et al. (2013) revealed that autotrophic nitrifying bacteria could utilize sulfonamide as the sole carbon and nitrogen source for metabolic activities to oxidize functional amino groups on the aromatic ring of sulfamethoxazole. Previous studies also reported that AOB could still degrade pharmaceuticals (e.g., atenolol, cephalaxin, and sulfadiazine) under ammonium-deficient conditions in enriched nitrifying sludge (Xu et al. 2017a; Wang et al. 2019a, 2019b).

Cometabolic biodegradation induced by AOB was found to be the dominant removal mechanism for ciprofloxacin. Ciprofloxacin is recalcitrant to be biodegraded in natural and artificial ecosystems (Girardi et al. 2011; Thuy & Loan 2014; Baginska et al. 2015). In this study, the higher removal efficiency and biodegradation rate in the presence of ammonium implied the biodegradation potential of enriched nitrifying sludge in removal of recalcitrant micropollutants. Since nitrite oxidizing bacteria (NOB) was reported to be irrelevant of micropollutant biodegradation (Yu et al. 2018), enhanced removal of antibiotics was mainly realized by AOB in the presence of substrate ammonium (Batt et al. 2006; Dorival-García et al. 2013; Ooi et al. 2018; Wang et al. 2019a, 2019b; Ramírez Muñoz et al. 2020). This enhanced capacity was related to AMO from AOB, which was able to degrade a wide range of aliphatic and aromatic compounds through cometabolism (Keener & Arp 1993; Skotnicka-Pitak et al. 2009; Ge et al. 2014). The positive linear correlation between AOR and ciprofloxacin degradation rate in this study also confirmed the presence of cometabolic biodegradation, which has been proved in previous studies on other classes of pharmaceuticals (Kassotaki et al. 2016; Xu et al. 2017a, 2017b; Wang et al. 2019a, 2019b). Higher removal of antibiotics (e.g., sulfamethoxazole, sulfadiazine, cephalaxin) was also achieved in the long-term nitrification SBR with average removal efficiency over 90% (Kassotaki et al. 2016; Wang et al. 2020). Further evaluation on the cometabolic biodegradation of ciprofloxacin will be performed in the continuous reactors.

Response of AOB during ciprofloxacin degradation

There was a gradual recovery process of AOR within initial 24 h of acclimation during cometabolic biodegradation of ciprofloxacin, which suggested that the initial 50 μg L⁻¹ of ciprofloxacin posed a temporary acute effect to the ammonium oxidation process. Nitrifying bacteria are more sensitive to antibiotics than other microorganisms in activated sludge (Schmidt et al. 2012; Song et al. 2015). Previous studies have also revealed that fluoroquinolones could induce DNA breakage and cell death (Drlica & Zhao 1997). There was a significant decrease in the abundance of AOB when exposed to ciprofloxacin at 350 ng L⁻¹ (Gonzalez-Martinez et al. 2014).

AOB likely neutralized this acute effect by triggering gene regulation to enable more amoA genes to be expressed and enhancing ammonium oxidation, allowing the recovery of AOB activity during the 24 h adaptation (Dorival-García et al. 2013; Wang et al. 2019a). Continuous decrease in AOR in the subsequent 120 h in the constant presence of ammonium was likely due to the competitive inhibition between cometabolic substrates (i.e., antibiotics) and growth substrates (i.e., ammonium) for the active AMO site (Sathyamoorthy et al. 2013). Besides, toxicity from biodegradation products might inhibit the activity of AOB, resulting in the decrease in AOR, which should be further verified. This notion was also reported in previous studies on biodegradation of sulfadiazine, cephalaxin, atenolol and acyclovir by enriched nitrifying sludge (Xu et al. 2017a, 2017b; Wang et al. 2019a, 2019b).

More evidence on the same trend of AMO activities as AOR also confirmed the inhibitory effect of ciprofloxacin on AOB. AMO is the responsible enzyme that can catalyze the cometabolic biotransformation of a large variety of organic pollutants (Tran et al. 2013). The elimination rate of trimethoprim, ibuprofen and naproxen increased with increasing ammonium loading rate, thereby increasing the biological activity of AOB, leading to more AMO production (Fernandez-Fontaina et al. 2012). When exposed to cephalaxin, the expression level of amoA gene was significantly up-regulated for the initial 24 h and then decreased to normal levels with cephalaxin consumed, revealing that AOB attempted to neutralize the toxicity of cephalaxin by generating more AMO with more mRNA (Wang et al. 2019a). Future studies are needed to investigate the underlying influencing mechanisms on AMO activities, which is significant to promote the cometabolic biodegradation.

CONCLUSION

This study investigated biodegradation of ciprofloxacin by an enriched nitrifying culture under different metabolic conditions and microbial responses to ciprofloxacin biodegradation products, which might provide the insights on improving existing wastewater treatment processes for removal of fluoroquinolones. The key findings are as follows:
1. The AOB cometabolic degradation was the main pathway for ciprofloxacin removal, while the contribution of metabolic biodegradation (AOB and heterotrophs) was relatively small.

2. There was a positive linear correlation between ciprofloxacin biodegradation rate and ammonium oxidation rate, confirming the dominance of AOB-induced cometabolic degradation pathway.

3. The continuous exposure of AOB to ciprofloxacin caused the decreases of AMO activity and AOR, demonstrating the inhibitory effect of ciprofloxacin on AOB.

4. The antibacterial activity upon ciprofloxacin was alleviated by the enriched nitrifying sludge through AOB-induced cometabolic biodegradation.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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