



Effect of ciprofloxacin on biological nitrogen and phosphorus removal from wastewater



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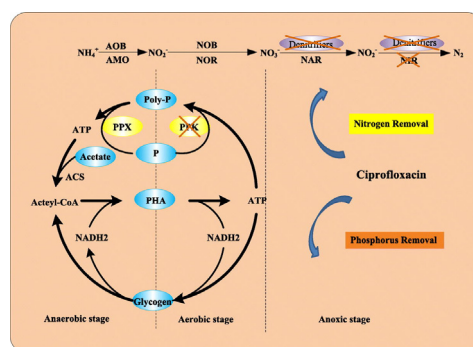
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HIGHLIGHTS

- The biological nutrient removal efficiency was inhibited by ciprofloxacin.
- The transformations of PHAs and glycogen were suppressed by ciprofloxacin addition.
- Ciprofloxacin inhibited NIR and PPK activities.
- Chronic-exposure ciprofloxacin affected the microbial community.

GRAPHICAL ABSTRACT



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ABSTRACT

In this work, both short-term and long-term experiments were therefore conducted to assess the effects of ciprofloxacin (0.2 and $2 \text{ mg} \cdot \text{L}^{-1}$) on wastewater nutrient removal. The results showed that both levels of ciprofloxacin had no acute and chronic adverse effects on the surface integrity and viability of activated sludge. Short-term exposure to all the ciprofloxacin levels induced negligible influences on wastewater nutrient removal. However, the prolonged exposure to ciprofloxacin decreased total phosphorus and nitrogen removal efficiencies from 96.8, 95.8% (control) to 91.7, 84.9% ($0.2 \text{ mg} \cdot \text{L}^{-1}$) and 90.5%, 80.2% ($2 \text{ mg} \cdot \text{L}^{-1}$), respectively. The mechanism study showed that ciprofloxacin exposure suppressed denitrification and phosphorus uptake processes. It was also found that ciprofloxacin affected the transformations of intracellular polyhydroxyalkanoates and glycogen in the oxic and anoxic stages. Moreover the activities of nitrite reductase and polyphosphate kinase were inhibited by the presence of ciprofloxacin. Further analysis with high-throughput sequencing revealed that compared with the control, the abundances of polyphosphate accumulating organisms, glycogen accumulating organisms and denitrifying bacteria in ciprofloxacin exposure reactors reduced, which were consistent with the decreased nutrient removal performance measured in these reactors.

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1. Introduction

Antibiotic is a kind of widespread pharmaceutical on disease prevention and control for both humans and animals (Kumar et al., 2012).

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They are delivered to animals and humans through feeding, injection, and medicine taking. Due to their high efficiency on bacteriostasis, antibiotics were abused largely. In the past few years, more and more antibiotics such as norfloxacin, sulfamethoxazole, nalidixic acid and trimethoprim have already been detected in wastewater effluent, river water and groundwater, and their concentrations are generally at nanograms, micrograms, or even milligram per liter (Kim et al., 2015; Dalmázio et al., 2007; Ayscough et al., 2000). Antibiotics were recently classified as a priority risk group due to their high toxicity to algae and bacteria at low concentrations and their potential to cause resistance among natural bacterial populations (Hermando et al., 2006). The occurrence of antibiotics in environment caused the rise in antibiotic resistance for pathogenic bacteria. As a consequence, its impacts on the population dynamics of bacteria and their antibiotic resistance genes in wastewater and river water drew much attention in the past few years (Huang et al., 2017).

Ciprofloxacin (CIP) is a broad spectrum fluoroquinolone widely used in both human and veterinary medicine. It is commonly utilized for the treatment of diseases such as pharyngitis, sinusitis, and earaches, as well as airway diseases, such as pneumonia and bronchitis (Coutu et al., 2013). It is recorded that the oral consumption of ciprofloxacin has increased by 30% in the last five years (Batt et al., 2007). Due to the abuse and the metabolism characteristic of CIP, it has been increasingly detected in wastewater (Li et al., 2011). The three major sources of CIP entering into wastewater are industry, hospital, and household. It is reported that the level of CIP in municipal wastewater is generally in the range of 100 to 300 ng·L⁻¹, while the CIP level in hospital wastewater is around 21 µg·L⁻¹ (Doorslaer et al., 2014). Wastewater CIP concentration discharged from CIP production facilities, however, could achieve at 4.9 mg·L⁻¹ (Babić et al., 2013). According to our survey on 12 wastewater treatment plants (WWTPs) of central China, which were located nearby either hospitals or CIP production facilities, CIP concentration in influent was in the range of 0.01–0.3 mg·L⁻¹.

CIP could affect DNA gyrase and topoisomerase IV of both Gram positive and Gram negative bacteria, thus preventing DNA replication and resulting in cell death (Martínez, 2008). Previous investigation showed that *Pseudomonas* sp., *Corynebacterium* sp., and *Micrococcus* sp. were sensitive to toxic effect of antibiotics (Akhter et al., 2013). As the last barriers before CIP entering into the aquatic environment, WWTPs are manipulated to deliberately culture a series of microorganisms such as nitrifiers, denitrifiers (D.B. Wang et al., 2017; Y.L. Wang et al., 2017), and polyphosphate accumulating organisms (PAOs) to remove pollutants (e.g., nitrogen and phosphorus) (Wang et al., 2012; Wang et al., 2016a; Y.G. Chen et al., 2014; H.B. Chen et al., 2014). The presence of CIP may also bring toxicity to these engineered microorganisms, thereby deteriorating the performances of WWTPs. Gonzalez-Martinez et al. reported that 350 ng L⁻¹ of CIP induced a deterioration of the partial-nitrification process from side-stream wastewater treatment. To date, however, the potential toxicity of CIP to mainstream wastewater nitrogen and phosphorus removal has not been reported.

The aim of this work is therefore to fully assess the potential toxicity of CIP to wastewater nitrogen and phosphorus removal. To gain more information, both municipal sewage relevant level (0.2 mg·L⁻¹) and CIP production facilities wastewater relevant level (2 mg·L⁻¹) of CIP were tested in this work. Firstly, the short-term and long-term effects of CIP on biological nitrogen and phosphorus removal were investigated. Then, the details of how CIP effects biological nitrogen and phosphorus removal were explored via analyzing the changes of the integrity of activated sludge surface, the transformations of metabolic intermediates, the activities of key enzymes, and the microbial communities. To the best of our knowledge, this is the first study fully evaluating the potential toxicity of CIP to wastewater treatment process, and the findings obtained here appeal engineers to pay more attention to the toxicity of

these emerging contaminants such as CIP to wastewater biological treatment process.

2. Materials and methods

2.1. The operation of parent reactor

A parent sequencing batch reactor with a working volume of 36 L was continuously operated. The seed sludge was obtained from the secondary sedimentation tank of a municipal wastewater treatment plant in Changsha, China. The CIP content in seed sludge was at non-detectable level. The reactor was maintained at 21 ± 1 °C and operated with 3 cycles daily. Each cycle consisted of a 1.5 h anaerobic phase, a 2.0 h oxic phase, and a 2.5 h anoxic phase, followed by 1 h settling, 20 min decanting, and 40 min idle phases. In the first 5 min of the anaerobic phase, 21 L synthetic wastewater with composition described as below was pumped into the reactor. A stirrer was used for mixing except for the settling, decanting, and idle phases. In the aerobic phase, air was provided into the reactor at a flow rate of 35 L·min⁻¹ by an aerator. The sludge retention time and hydraulic retention time were maintained at approximately 12 d and 11 h, respectively. The influent pH was adjusted to 7.4 by adding 2 M HCl or 2 M NaOH. After about 50 d operation, stable operation was achieved in this parent reactor, and then the sludge was withdrawn for the following toxicity tests.

The concentrations of chemical oxygen demand (COD), ammonium (NH₄⁺-N) and soluble orthophosphorus (SOP) were controlled at approximately 300, 30, and 10 mg·L⁻¹ respectively, thus the detailed composition of synthetic wastewater was as below (mg·L⁻¹): 384.6 sodium acetate, 14.62 KH₂PO₄ and 49.03 K₂HPO₄·3H₂O, 114.4 NH₄Cl, 5 mg CaCl₂, 10 mg MgSO₄·7H₂O and 0.5 mL of trace-element solution. The trace metals solution consisted of (g·L⁻¹): 1.50 FeCl₃·6H₂O, 0.06 Na₂MoO₄·2H₂O, 0.03 CuSO₄·5H₂O, 0.12 MnCl₂·4H₂O, 0.15 CoCl₂·6H₂O, 0.12 ZnSO₄·7H₂O, 0.15 H₃BO₃, 0.18 KI and 10 ethylenediamine tetra-acetic acid (Wang et al., 2008).

2.2. Acute and chronic exposure to CIP

For acute test, the experiments were implemented in 3 replicate reactors with working volume of 3 L each. A total of 9 L of activated sludge mixture was taken from the parent reactor at the end of anoxic phase and divided equally into the three reactors. After settling and decanting 2 L of supernatant, reactors I and II received wastewaters containing 0.2 mg·L⁻¹ and 2 mg·L⁻¹ of CIP, while reactor III received wastewater containing no CIP and was set as the control. Except CIP level, other composition and concentration of the synthetic wastewaters were the same with the parent reactor. All the operational conditions were also the same to the parent reactor. The effluent of ammonium-nitrogen (NH₄⁺-N), nitrite-nitrogen (NO₂⁻-N), nitrate-nitrogen (NO₃⁻-N), polyhydroxyalkanoates (PHA), glycogen, sludge viability, and LDH release (cell membrane damage marker) were measured every 30 min during one cycle.

For chronic experiment, all the reactors carried out above were ceaselessly operated to analyze the possible chronic effect of CIP. The operational conditions of the three reactors were respectively the same to those in the acute test. The effluent concentration of P, NH₄⁺-N, NO₂⁻-N, NO₃⁻-N was recorded daily. After 60 d-exposure, all reactors achieved steady-state operation, then the transformation of NH₄⁺-N, P, NO₂⁻-N, NO₃⁻-N, PHA, glycogen, sludge viability, LDH release, and the activities of key enzymes relevant to biological nitrogen and phosphorus removal (i.e., ammonia monooxygenase (AMO), nitrite oxidoreductase (NOR), nitrate reductase (NAR), nitrite reductase (NIR), exopolyphosphatase kinase (PPX) and polyphosphate kinase (PPK)) during one cycle were determined. Moreover, the effect of CIP on the bacterial community structure was also explored by high-throughput sequencing.

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