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Short-term effects of ciprofloxacin on enhanced biological phosphorus removal based on anaerobic and aerobic metabolism

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ABSTRACT

Understanding the effects of antibiotics on enhanced biological phosphorus removal (EBPR), which has been widely accepted as the most economical and sustainable process for removing phosphorus from wastewater to control eutrophication problems, is very important. The potential role of ciprofloxacin (CIP) in inhibiting wastewater phosphorus removal during short-term exposure and the associated mechanisms were investigated in this study. The results showed that the inhibitory effect of CIP on phosphorus removal efficiency was dose- and time-dependent. Total extracellular polymeric substances and polysaccharide (PS) production were immediately enhanced as the CIP concentration increased, implying that PS was secreted to resist toxicity. However, these parameters decreased with increasing reaction cycles. Reactive oxygen species (ROS) increased remarkably during the aerobic phase, resulting in a decreased oxygen uptake rate. Moreover, variations in stoichiometric parameters and kinetic rates showed that anaerobic and aerobic metabolism was affected by CIP, with the aerobic metabolism of phosphate-accumulating organisms being more sensitive to CIP than anaerobic metabolism, probably due to the increase in ROS. Furthermore, glycogen production was strongly inhibited by CIP, and the tricarboxylic acid cycle might be involved in anaerobic metabolism. The results of this study offer insights into the short-term effects of CIP on biological phosphorus removal from the view of anaerobic and aerobic metabolism, which enables further study on control strategies for reducing CIP inhibition and maximizing the reliability and efficiency of EBPR.

Keywords: Antibiotics; Biological phosphorus removal; Reactive oxygen species; Extracellular polymeric substances; Stoichiometric parameters

1. Introduction

Antibiotics have been widely used in humans and animals since the discovery of penicillin in 1929 [1,2]. Global antibiotic consumption increased by 65% (21.1–34.8 billion defined daily doses) between 2000 and 2015 and is projected to increase by 15% between 2015 and 2030 [3]. Additionally, the global consumption of antimicrobials in food animals was estimated at 131,109 tons in 2013 and is projected to reach 200,235 tons by 2030 [4]. Unfortunately, only a small fraction of antibiotics are metabolized or absorbed by the body, and a high percentage (50%–90%) of consumed antibiotics are excreted via urine and feces [5]. As a result, the released antibiotics are transported into wastewater treatment plants (WWTPs) through municipal pipelines. Although many technologies, such as advanced oxidation, adsorption, and bioelectrochemical systems, have been studied to remove antibiotics [6–8], WWTPs are

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not specifically designed and operated to remove antibiotics, and various groups of antibiotics have been frequently detected in WWTPs [5,9–11]. The presence of antibiotics in wastewater treatment systems has adverse effects on microorganisms, leading to a decline in wastewater treatment capacity [12–14]. Therefore, it is very important to understand the effects of antibiotics on biological nutrient removal.

Excessive phosphorus in aquatic environments induces eutrophication, which has become a significant water quality problem worldwide. Chemical or biological processes are usually used for phosphorus removal from wastewater. Enhanced biological phosphorus removal (EBPR) has been widely accepted as the most economical and sustainable process for removing phosphorus (P) from wastewater to control eutrophication problems [15,16]. The performance of EBPR systems operating in an anaerobic/aerobic configuration depends on the enrichment of phosphate-accumulating organisms (PAOs) [17]. EBPR systems are relatively fragile in that toxic compounds are harmful to the EBPR process and could result in its deterioration [15,18]. Previous studies have shown that EBPR systems can also be influenced by antibiotics [19–21]. The P removal efficiency in an EBPR system was reduced to 34.6% and 0.0% after exposure to erythromycin and oxytetracycline at 10 mg/L for 24 h [19]. However, research on the effects of antibiotics on P removal in EBPR systems is still limited.

Fluoroquinolones (FQs) are wide-spectrum antibacterial agents that are being increasingly used in hospitals, households, and veterinary applications [22,23]. Ciprofloxacin (CIP) is one of the most widely used antibiotics among all fluoroquinolone antibiotics [1]. Yi et al. [11] found that the CIP concentration in the influent of 12 WWTPs that were located near either hospitals or CIP production facilities was 0.01–0.3 mg/L. High concentrations of CIP have also been reported in effluent from hospitals (0.9 mg/L) and municipal wastewater (0.25 mg/L) [5]. CIP is toxic to microorganisms in activated sludge systems. It was found that P removal was inhibited after the exposure of CIP to a biological nutrient removal system, and it was even more severely inhibited than chemical oxygen demand (COD) removal and nitrification [11,13]. Different stoichiometric parameters and kinetic rates can provide important information regarding not only EBPR activity and efficiency but also metabolism [17,24]. However, how CIP affects P removal in the EBPR system, especially from the perspective of anaerobic and aerobic metabolism, has not been determined and warrants further study.

To address these issues and maximize the reliability and efficiency of the EBPR process, a study of the effects of CIP on biological P removal based on anaerobic and aerobic metabolism is greatly needed. The aim of this work is to fully assess the potential inhibitory effects of CIP on wastewater phosphorus removal during short-term exposure by investigating the variations in EBPR performance with changes in CIP concentration and reaction cycle. Furthermore, the effects of CIP on phosphorus removal were determined by investigating anaerobic and aerobic metabolism based on variations in stoichiometric parameters and kinetic rates. The effects of CIP on the production of extracellular polymeric substances (EPS) and reactive oxygen species (ROS) were also investigated to further reveal the mechanism by which CIP inhibits the removal of phosphorus from wastewater. Thus, this study is relevant to improve our knowledge about the short-term effects of CIP on microbial metabolism in EBPR systems and to further comprehensively understand the impact of antibiotics, which is necessary for better understanding and optimizing the performance of the process.

2. Materials and methods

2.1. Parent sequencing batch reactor operation

Seed sludge was collected from a WWTP in Hangzhou, China. Five 10-L laboratory-scale anaerobic-aerobic sequencing batch reactors (SBRs) fed with 2.5 L of synthetic wastewater were used to enrich PAOs. The reactors were operated at 20°C ± 1°C for four cycles each day. The sludge retention time (SRT) and hydraulic retention time (HRT) was controlled at 10 d and 24 h, respectively. Each cycle (6 h) consisted of the following steps: 5 min for feeding synthetic wastewater, 120 min for the anaerobic phase, 150 min for the aerobic phase, 60 min for sedimentation, 5 min for extraction and 20 min for idling. After three months, the reactors were running in a steady state with mixed liquor suspended solids (MLSS) concentrations of 2,500–3,000 mg/L.

Synthetic wastewater containing 32 mg P/L phosphate and 1,000 mg COD/L (acetate) was prepared for the experiment. The synthetic wastewater consisted of (per liter water) 1.28 g of CH_3COONa, 0.229 g of NH_4Cl, 0.07 g of KH_2PO_4, 0.0895 g of K_2HPO_4, 0.09 g of $MgSO_4$, 0.022 g of $CaCl_2$, 0.02 g of allylthiourea (ATU) and 0.6 mL of trace element solution. The trace element solution was prepared according to the protocol described by Hu et al. [19].

2.2. Batch experiments

Batch experiments were conducted in six 5-L laboratory-scale anaerobic-aerobic SBRs, and the operation conditions and synthetic wastewater were consistent with the parent reactors. A total of 30 L of activated sludge mixture was taken from the parent reactors at the end of the aerobic phase and divided equally into 6 reactors. As high concentrations of CIP were observed in wastewater, 0.05, 0.1, 0.2, 0.5, and 1 mg/L CIP were used to investigate the acute effects in this study. CIP, which was purchased from Shanghai Aladdin Biochemical Technology Co., Ltd., China, was added to initial calculated concentrations of 0.05, 0.1, 0.2, 0.5, and 1 mg/L and labeled R1, R2, R3, R4, and R5, respectively. A reactor with no CIP was treated as a control and labeled R0.

Mixed liquor samples were taken at intervals for analysis of polyhydroxyalkanoate (PHA), glycogen (Gly), orthophosphate, and acetate. Before being analyzed for orthophosphate and acetate, samples were immediately filtered through 0.45-μm Millipore filters. Mixed liquor samples at the beginning of each cycle and at the end of the anaerobic and aerobic phases were taken for analysis of EPS and ROS.

2.3. Analytical methods

The MLSS and orthophosphate were analyzed according to standard methods [25].

Acetate was determined using a GC system (Nexis GC-2030, Shimadzu, Japan) combined with a flame ionization detector (FID). Water samples were filtered through a 0.22-μM membrane filter, and 1/10 of 3% phosphoric acid was added before determination. The gas chromatographic column was DB-WAX (30 m × 0.32 mm). The injection port and the detector were maintained at 200°C and 220°C, respectively. The GC system (Nexis GC-2030, Shimadzu, Japan) was also used to determine the amount of poly-β-hydroxybutyrate (PHB), poly-β-hydroxyvalerate (PHV) and poly-β-hydroxy-2-methylvalerate (PH2MV) [26]. The total PHA amount was calculated as the sum of measured PHB, PHV, and PH2MV. For the glycogen analysis, preweighed samples of lyophilized sludge were added to 5 mL of 0.6 M HCl and digested at 100°C for 6 h. After cooling, the samples were centrifuged, and 1 mL of the supernatant liquid was removed for analysis by the anthrone method originally introduced by Dreywood [27].

EPS were extracted by the cation exchange resin method [28]. Generally, proteins (PN) and polysaccharides (PS) (75%–90%) are the dominant components of EPS, and humic acid (HA) may also be a key component with small amounts of nucleic acids, uronic acids and lipids [29–31]. Therefore, the PN, PS and HA in EPS were measured in this study. PN were measured by a Modified BCA Protein Assay Kit (Sangon Biotech, China), PS were measured using the anthrone method [27], and HA was measured by spectrophotometry at 254 nm using a calibration curve [32].

ROS were determined by a Reactive Oxygen Species Assay Kit (S0033, Beyotime, China) according to the manufacturer's instructions. Activated sludge was stimulated with medium containing 10 μmol/L dichloro-dihydrofluorescein diacetate (DCFH-DA) for 20 min at 37°C. The sludge was slightly shaken every 5 min. After removing the medium and washing the sludge with phosphate-buffered saline (PBS), the sludge was collected, and the fluorescence intensity of each sample was examined using a fluorescence spectrophotometer (F-4600, Hitachi, Japan).

A respirometer (RSA PF-8000, USA) was used to determine the oxygen uptake rate (OUR). A total of 2 L of activated sludge mixture was taken from the parent reactors at the end of the aerobic phase and divided equally into 5 bottles, and synthetic wastewater was consistent with the parent reactor contents. CIP was added to initial calculated concentrations of 0, 0.1, 0.2, 0.5, and 1 mg/L. After 120 min of the anaerobic phase, the OUR during the aerobic phase was measured.

The inhibition of P removal efficiency was calculated by Eq. (1).

$$\text{Inhibition} = \frac{\left(S_{R0} - S_{Ri}\right)}{S_{R0}} \quad (1)$$

where *S*R0 is the P removal efficiency of R0; S_{Ri} is the P removal efficiency of Ri (i = 1, 2, 3, 4, 5).

3. Results and discussion

3.1. Effects of CIP on P removal

The SBRs functioned in a steady-state and performed >99% P removal when the batch experiments were carried out. CIP exhibited inhibitory effects on P removal during shock loading (Fig. 1). In the first cycle, the amounts of orthophosphate released in the anaerobic phase and taken up in the aerobic phase were similar in all test reactors. Thereafter, anaerobic P release and aerobic P uptake gradually decreased over time, and higher CIP exposure was associated with more severe decreases. Notably, the inhibitory effect of CIP on P removal efficiency was dose- and time-dependent. The P removal efficiencies in the third cycle in R2, R3, R4 and R5 declined to 95.4%, 93.5%, 90.0% and 89.8%, respectively, and decreased even further to 92.5%, 88.1%, 78.4% and 72.4%, respectively, in the fourth cycle. Furthermore, the inhibition of P removal efficiency followed a parabola with increasing CIP concentration in the fourth cycle (Fig. 2a).

The maximum P release rates with additional CIP were all lower than that in R0, which is reflected in the maximum P release rates that were normalized against R0, which were all lower than 1 (Fig. 2b). A similar phenomenon was also found for the maximum P uptake rates (Fig. 2b). Notably, the maximum P uptake rate decreased more drastically than the maximum P release rate, indicating that aerobic phosphate uptake was inhibited more sharply than anaerobic phosphate release. Similarly, by investigating the importance in the projection values for concentrations of P uptake and P release, Wu et al. [21] found that P uptake was more sensitive than P release under short-term antibiotic pressure.

Notably, the consumption of acetate, which is the main carbon source that can be taken up by PAOs and converted into intracellular PHA, was only slightly affected by CIP. Even when the concentration of CIP was as high as 1 mg/L in the fourth cycle, acetate was still completely taken up within an hour. There may be another group of microorganisms known as glycogen-accumulating organisms (GAOs) that can compete with PAOs for the anaerobic uptake of volatile fatty acids (VFA) without contributing to P removal. The activities of both PAOs and GAOs may be affected by CIP, and the effects of CIP on PAOs and GAOs may even be different. P release was inhibited by CIP without an obvious difference in acetate uptake. The different variations in phosphate and acetate led to a clear decrease in the $P_{release}$/VFA_{uptake} ratio with increasing CIP concentration in the fourth cycle (Fig. 2c). The value in R5 decreased by 27.9% compared to that in R0 in the fourth cycle. The decreased value of the $P_{release}$/VFA_{uptake} ratio suggests that EBPR activity was limited by the presence of CIP and that exposure to more CIP was associated with more severe inhibition.

3.2. Effects of CIP on glycogen and PHA

PHA accumulation and glycogen degradation occurred during the anaerobic phase. During the aerobic phase, PHA utilization and glycogen replenishment occurred concurrently. In the reactor without CIP, PHB was approximately 90% of all the PHA, a small amount of PHV was synthesized, and no PH2MV was produced. This composition is usually reported in EBPR systems fed acetate [33,34].

Similar to P release and P uptake, the amount of PHA and glycogen varied during the anerobic and aerobic phases and gradually decreased with time, and greater CIP exposure was associated with more severe decreases (Figs. 3 and 4). Moreover, the degradation of PHA and the

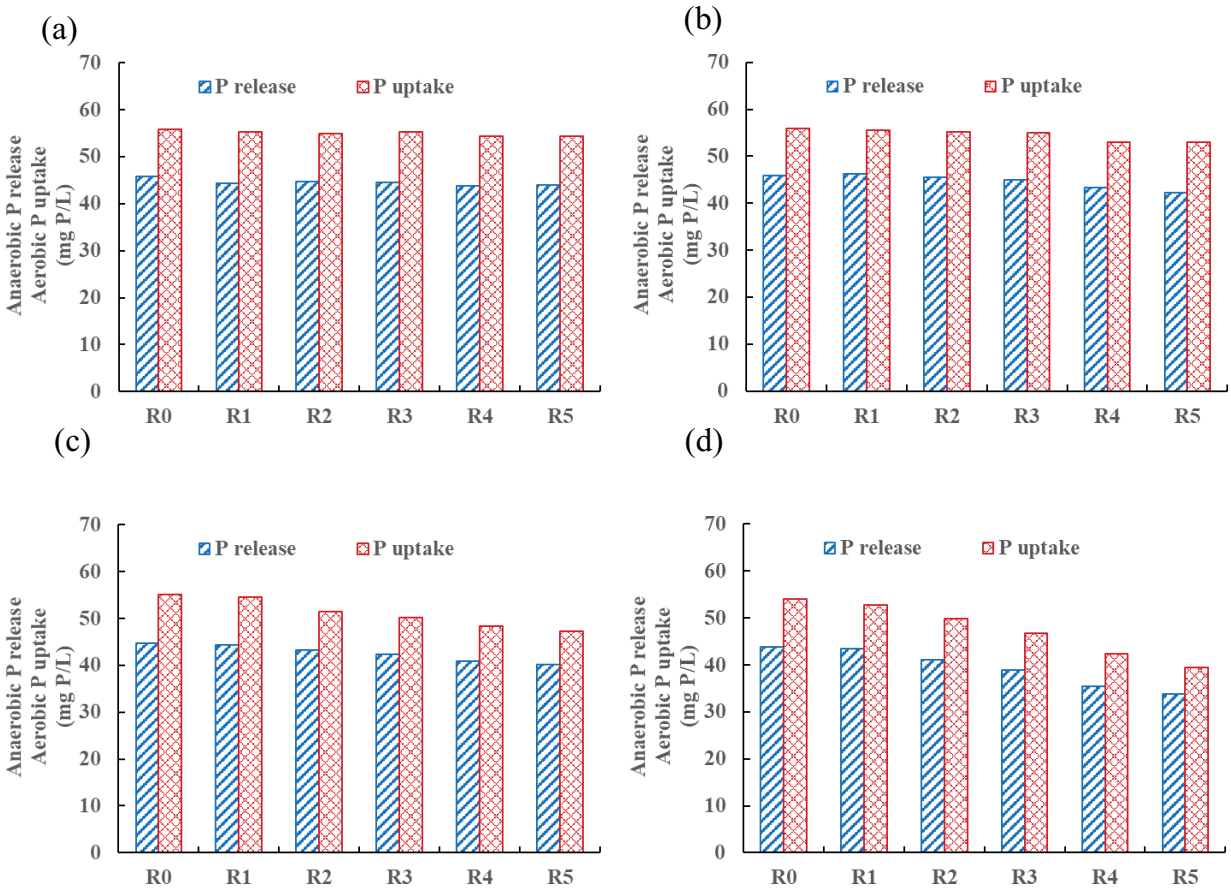

Fig. 1. Effects of CIP on anaerobic P release and aerobic P uptake in the first (a), second (b), third (c) and fourth cycles (d).

accumulation of glycogen during the aerobic phase showed a more severe decrease than the accumulation of PHA and the degradation of glycogen during the anaerobic phase. In the fourth cycle, the aerobic PHA consumption and glycogen replenishment in R5 compared to those in R0 decreased by 33.3% and 53.1%, respectively, whereas the anaerobic PHA accumulation and glycogen degradation in R5 compared to those in R0 decreased by 25.7% and 46.8%, respectively. These results indicated that the aerobic metabolism of PAOs was inhibited more strongly than was anaerobic metabolism. In addition, aerobic glycogen replenishment was suppressed more strongly than was aerobic PHA consumption. Compared to that in R0, the PHA consumption at the aerobic phase in the fourth cycle of R1, R2, R3, R4, and R5 decreased by 7.4%, 9.6%, 14.8%, 27.7% and 33.3%, respectively. Glycogen replenishment in the fourth cycle of R1, R2, R3, R4, and R5 decreased by 16.7%, 25.8%, 27.6%, 48.7% and 53.1%, respectively.

Often, the $PHA_{synthesized}/VFA_{uptake}$, $Gly_{degraded}/VFA_{uptake}$, $Gly_{synthesized}/PHA_{degraded}$, and $P_{uptake}/PHA_{degraded}$ ratios are the main stoichiometric parameters important for assessing the anaerobic and aerobic stoichiometry of the EBPR processes. Further investigations showed that the anaerobic $PHA_{synthesized}/VFA_{uptake}$ ratios in the first cycle were affected slightly by CIP, while these ratios declined notably with the increase in CIP concentration in the fourth cycle, decreasing from 1.01 C mmol/C mmol in R0 to 0.69 C mmol/C mmol in R5 (Fig. 5a). A similar variation trend was found for the anaerobic ratios of $Gly_{degraded}/VFA_{uptake}$ and aerobic ratios of $Gly_{synthesized}/PHA_{degraded}$ (Figs. 5b and c). The ratios of $Gly_{degraded}/VFA_{uptake}$ in the fourth cycle decreased from 0.34 C mmol/C mmol in R0 to 0.18 C mmol/C mmol in R5. The ratios of $Gly_{synthesized}/PHA_{degraded}$ in the fourth cycle decreased remarkably from 0.25 C mmol/C mmol in R0 to 0.14 C mmol/C mmol in R5. A slight decrease in the aerobic ratios of $P_{uptake}/PHA_{degraded}$ with increasing CIP concentration in the first cycle was observed, and a similar variation trend was also observed in the fourth cycle (Fig. 5d). These results suggest that the inhibition of the ratios of $PHA_{synthesized}/VFA_{uptake}$, $Gly_{degraded}/VFA_{uptake}$, and $Gly_{synthesized}/PHA_{degraded}$ by CIP was dose- and time-dependent.

Anaerobic hydrolysis of glycogen provides the required reducing power driving VFA to PHA conversions [35]. In the aerobic phase, PAOs oxidize the PHA stored in the anaerobic phase as a carbon and energy source to (i) take up and store phosphorus, (ii) replenish the intracellular glycogen pool, (iii) support aerobic growth of PAOs, and (iv) cover the aerobic maintenance energy needs of PAOs [36]. The variations in the ratios of $Gly_{synthesized}/PHA_{degraded}$ and $P_{uptake}/PHA_{degraded}$ indicate that aerobic glycogen production can be strongly inhibited by CIP with a slight influence of the utilization of PHA for P uptake. On the other hand, the utilization of PHA for aerobic growth of

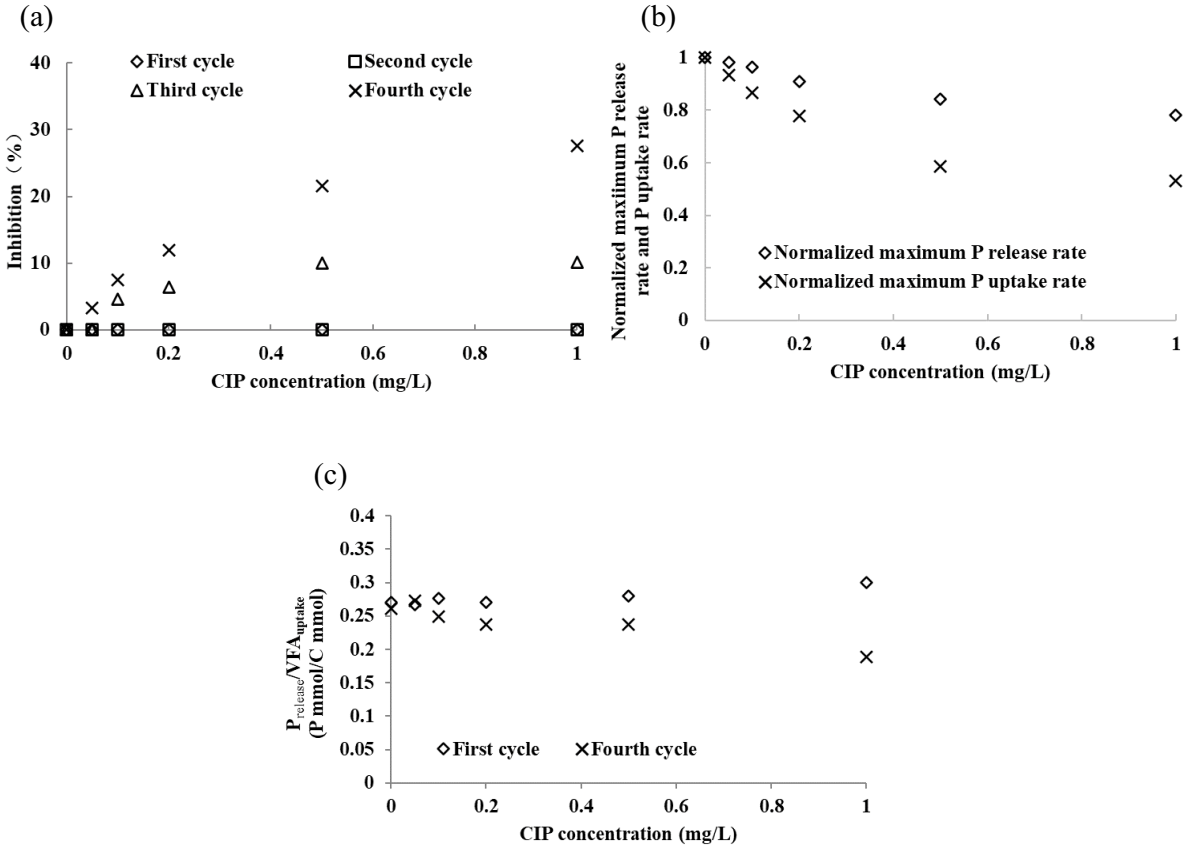

Fig. 2. The inhibition of P removal efficiency by CIP in the four cycles (a), the normalized maximum P release and P uptake rates (normalized against R_0) in the fourth cycle (b), and the $P_{release}/VFA_{uptake}$ ratios in the first and fourth cycles (c).

PAOs may be inhibited because CIP could affect DNA gyrase and topoisomerase IV of bacteria, thereby preventing DNA replication [37]. Moreover, lower production of glycogen in the aerobic phase resulted in limited glycogen for the anaerobic phase. Acetate can still be taken up anaerobically to generate reducing power under limited glycogen conditions, and the production of reducing power from acetate is likely through the tricarboxylic acid (TCA) cycle [24,38,39]. It was predicted that 10%–11% of acetyl-CoA was oxidized through the TCA cycle to generate 65%–100% of the reducing power under glycogen-limited conditions, but only 4% of acetyl-CoA passed through the TCA cycle to produce 25% of the total reducing power for PHA synthesis under normal conditions [24]. The proportion of TCA cycle involvement also depends on the availability of degradable glycogen [24]. The results obtained in this study reveal that the TCA cycle might be involved when glycogen content decreases, leading to lower glycogen consumption and PHA production per acetate uptaken. It was also hypothesized that TCA cycle utilization is associated with a higher $P_{release}/VFA_{uptake}$ ratio to compensate for the required energy, since less energy is obtained from glycogen breakdown [24]. The slightly increasing $P_{release}/VFA_{uptake}$ ratio with increasing CIP concentration in the first cycle (0.27 P mmol/C mmol in R0 to 0.30 P mmol/C mmol in R5) observed in this study was in accordance with this theory. The decreasing $P_{release}/VFA_{uptake}$ ratio with increasing CIP concentration in the fourth cycle might be primarily caused by the inhibitory effects of CIP. The phenomenon that P release, PHA accumulation and glycogen degradation were all inhibited while acetate uptake was not obviously affected by CIP might result from flexible metabolic operation under anaerobic conditions [39,40]. As mentioned in [39], metabolism could favor pathways with higher ATP (adenosine triphosphate) yield in the case of polyphosphate limitation. A detailed study is necessary to further determine PAO metabolism after exposure to CIP.

3.3. Effects of CIP on EPS

As shown in Fig. 6, the total EPS production varied with the operational conditions. The EPS concentrations at the end of the aerobic phase were higher than those at the end of the anaerobic phase. This occurred because EPS is a complex high-molecular-weight mixture of polymers produced by microorganisms in EBPR at the aerobic stage [30]. The results showed that the total EPS immediately increased with increasing CIP concentration in the first cycle, from 188 mg/L in R0 to 200 mg/L in R5 at the end of the aerobic phase. However, the increase was blocked, and the total EPS decreased with increasing CIP concentration in the third and fourth cycles. The roles of EPS in the EBPR system have gained increasing attention, and it is known that microbial

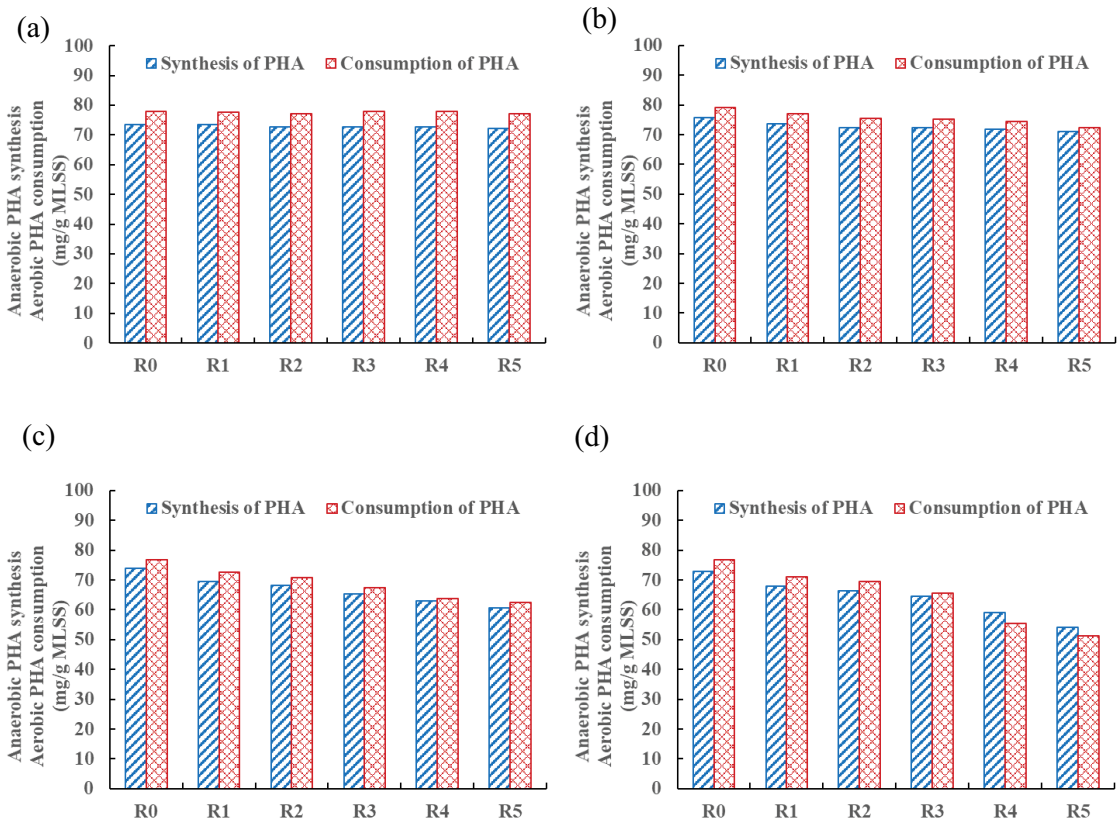

Fig. 3. Amount of PHA synthesis and consumption in the first (a), second (b), third (c) and fourth cycles (d).

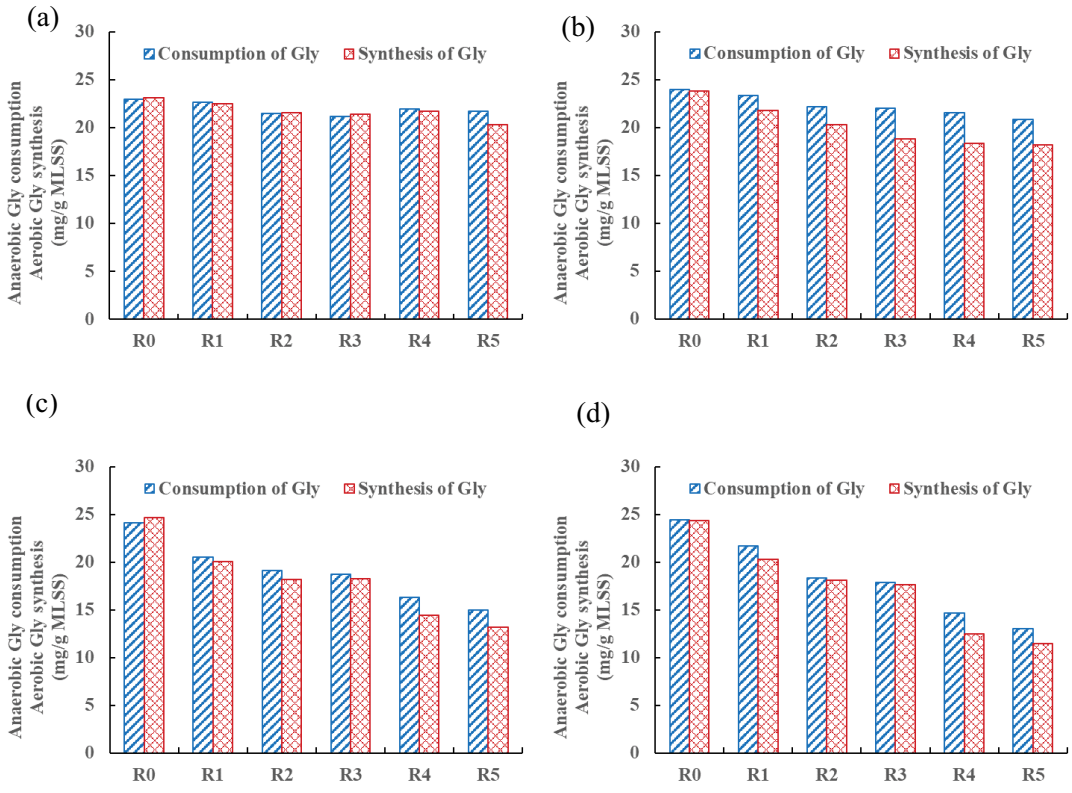

Fig. 4. Amount of Gly synthesis and consumption in the first (a), second (b), third (c) and fourth cycles (d).

cells produce more EPS to protect themselves from harsh environments in the presence of toxic substances [30,41]. Therefore, the addition of CIP stimulated the production of EPS, but the effect on the promotion of EPS production became less significant as the inhibitory effect of CIP increased. The EPS production enhancement may be one reason for the stable phosphate removal efficiency in all the test reactors in the first and second cycles.

Further research showed that the EPS composition was influenced by the presence of CIP. The PS concentration increased with increasing CIP concentration once CIP was added but decreased with more cycles. However, the PN concentration at the end of the anaerobic phase in the first cycle decreased slightly with increasing CIP concentration, and it increased slightly in the consequent aerobic phase but then decreased. Compared with the PN concentration in R0, the PN concentration at the end of the aerobic phase in the fourth cycle decreased by 2.1%, 3.6%, 5.4%, 6.4% and 10.6% in R1, R2, R3, R4, and R5, respectively. In contrast to the PS concentrations, the HA concentrations were only slightly affected by the presence of CIP in the first cycle. The HA concentration only slightly increased with functional time. The HA concentrations in R1, R2, R3, R4, and R5 were higher than that in R0 in the fourth cycle. The HA concentration also increased when other kinds of antibiotics were added to activated sludge systems [42]. However, the mechanism was not investigated and needs further study. It is clear that the increase in the total EPS in the first cycle was mainly caused by the increase in PS, and the decrease in the total EPS in the fourth cycle was caused by the decreases in PS and PN.

3.4. Effects of CIP on ROS

ROS are highly reactive atoms or molecules found in all aerobic biological organisms, and ROS generation in bacterial cells is primarily associated with undesirable electron transfers to O_2 during aerobic respiration and metabolism [43]. ROS cause structural damage to proteins, enzymes, nucleic acids, and membranes, resulting in cell death when the level of ROS exceeds an organism's detoxification and repair capabilities [44,45]. Antibiotics can influence the redox balance in bacteria, and ROS production is one of the lethal factors of bactericidal antibiotics [46,47]. In this study, the ROS gradually increased with functional time and varied with the operation conditions (Fig. 7). The fluorescence intensity of ROS increased slightly during the anaerobic phase and remarkably during the aerobic phase, and the increased CIP exposure was associated with higher ROS fluorescence intensity. Compared to the level in R0, the ROS in R1, R2, R3, R4, and R5 increased by 16.1%, 15.9%, 40.6%, 42.8%, and 49.5% at the end of the aerobic phase in the fourth cycle. The results obtained in this study indicated that CIP induces ROS production mostly during the aerobic phase, and increased CIP exposure was associated with increased ROS production. Thus, aerobic phosphate uptake was inhibited more significantly than anaerobic phosphate release.

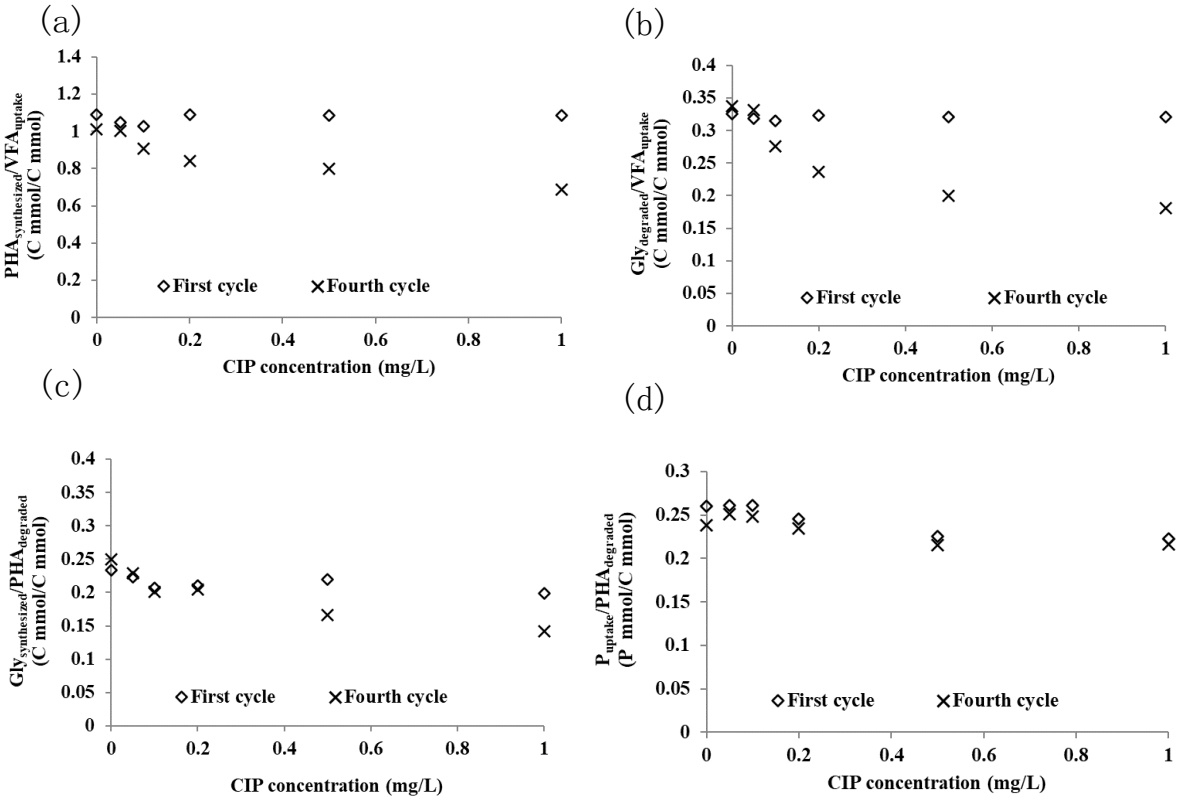

Fig. 5. $PHA_{synthesized}/VFA_{uptake}$ (a), $Gly_{degraded}/VFA_{uptake}$ (b), $Gly_{synthesized}/PHA_{degraded}$ (c), and $P_{uptake}/PHA_{degraded}$ (d) ratios were observed in the first and fourth cycles with different CIP concentrations.

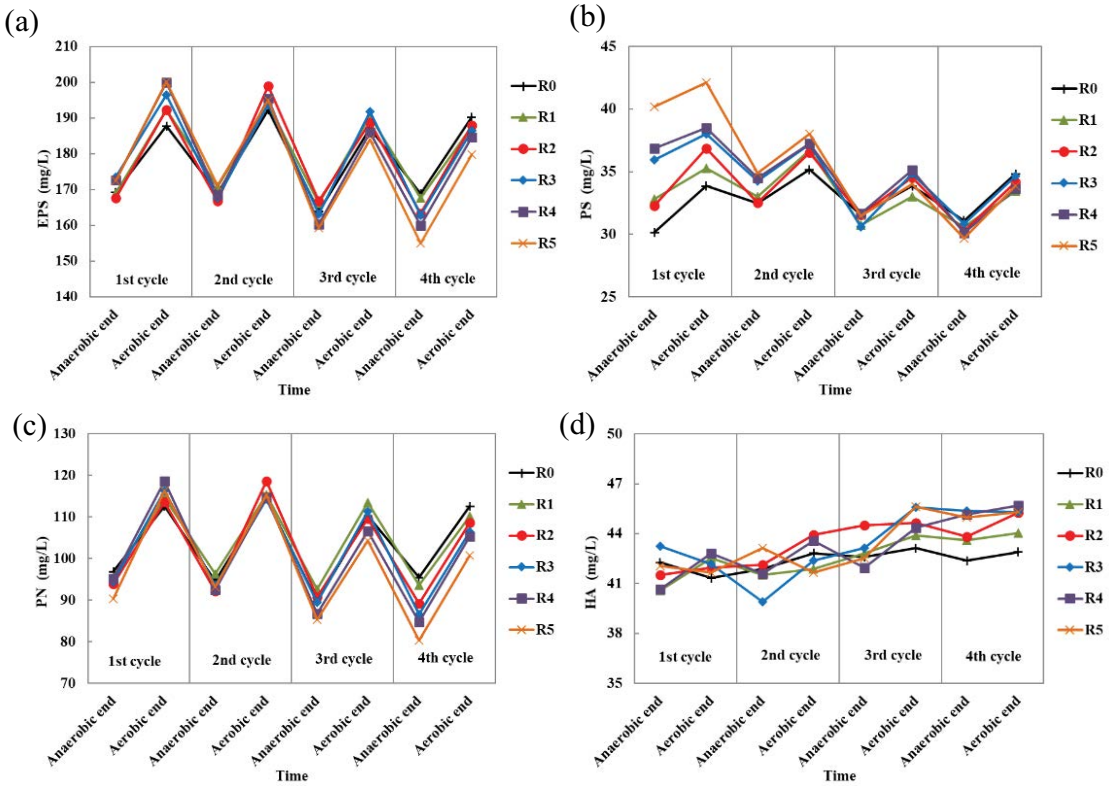

Fig. 6. Total EPS (a) concentration and composition (PS (b), PN (c), and HA (d)) at the end of the anaerobic and aerobic phases in each cycle.

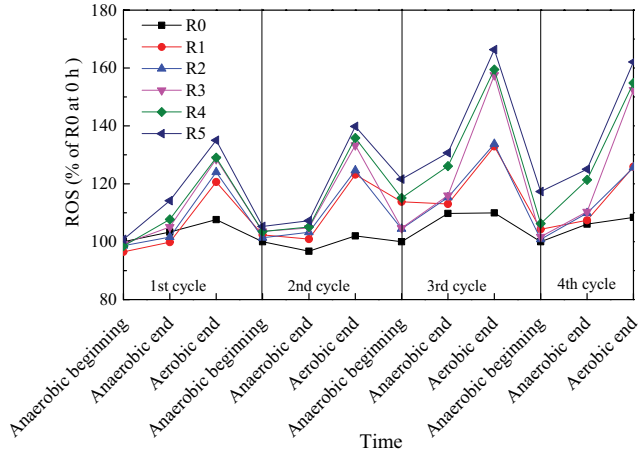

Fig. 7. Variations in ROS in the four cycles.

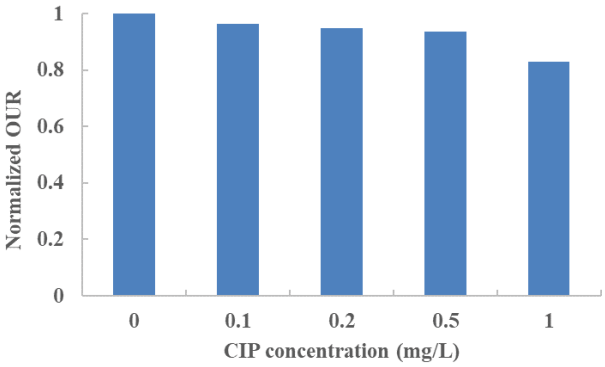

Fig. 8. The variations in the OUR in the first cycle.

Notably, the OUR decreased in the reactors exposed to CIP compared with the reactor that was not exposed to CIP, and greater CIP exposure was associated with lower OUR (Fig. 8). Compared to the reactor without additional CIP, in the reactors with CIP, the average OUR values for 0.1, 0.2, 0.5, and 1 mg/L CIP decreased by 3.6%, 5.2%, 6.5%, and 17.2%, respectively, during the 2.5-h aeration. The decreased OUR values were probably caused by increased ROS, since antibiotics that increased ROS generation in cells inhibited the rate of oxygen consumption in the cells [48]. Compared to the rates of P uptake, PHA consumption and glycogen production in the first cycle, the OUR was more notably influenced by CIP. This is because the organisms' growth rate may be inhibited by CIP. This hypothesis is supported by the observation that the nongrowing (lag) phase protected bacteria from antibiotics and extended lag reduced the killing rate [49].

4. Conclusion

This study demonstrated that the inhibitory effect of CIP on P removal efficiency was dose- and time-dependent.

The PS concentration immediately increased with increasing CIP concentration, possibly causing the stable phosphate removal efficiency in the first two cycles. In the fourth cycle, the P removal efficiency declined to 92.5%, 88.1%, 78.4% and 72.4% at 0.1, 0.2, 0.5, and 1 mg/L CIP, respectively. The maximum P uptake rate decreased more drastically than the maximum P release rate. PHA degradation and glycogen accumulation decreased more severely during the aerobic phase than variations in PHA and glycogen during the anaerobic phase. Thus, it can be concluded that the aerobic metabolism of PAOs was more sensitive than anaerobic metabolism to CIP, which was supported by the remarkable ROS increase during the aerobic phase. Notably, aerobic glycogen production was strongly inhibited by CIP, and the TCA cycle might be involved in anaerobic metabolism under limited glycogen conditions. This study mainly focused on the short-term effects of CIP, and determining the long-term effects of CIP requires further study considering anaerobic and aerobic metabolism and bacterial community structure.

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