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Effects of chlortetracycline on biological nutrient removal from wastewater

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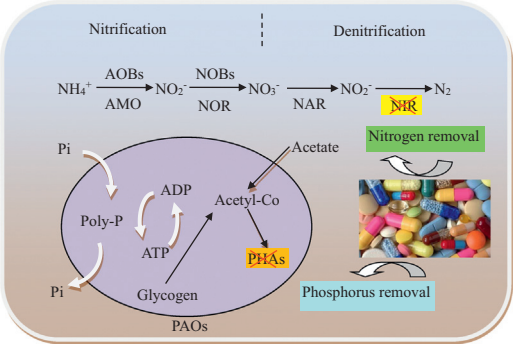
HIGHLIGHTS

- The high level of CTC could affect nitrogen and phosphorus removal.
- Microbial enzymatic activities were analyzed at different CTC concentration.
- Nitrogen and phosphorus removal rate was related to microbial enzymatic activity.
- The transformations of PHAs and glycogen were suppressed by CTC addition.

GRAPHICAL ABSTRACT

This study showed the long-term exposure to mg·L^{-1} CTC inhibited denitrification and phosphorus uptake by affecting the transformations of PHAs and glycogen and decreasing the activity of NIR.



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ABSTRACT

Due to the widespread use of antibiotics in healthcare and livestock production, antibiotic resistance genes and residual antimicrobials would enter environment and further discharge into the municipal sewage system. The objective of this work was to explore the potential effect of chlortetracycline (CTC) on biological nutrient removal from wastewater. Thus, the effects of CTC on biological phosphorus and nitrogen removal were investigated with respect to the viability of bacteria, the activities of key metabolic enzymes, and the transformations of intermediate metabolites. Results showed that the presence of 0.1 mg·L^{-1} CTC did not show any impact on biological phosphorus and nitrogen removal. Nevertheless, the long-term exposure to 1 and 10 mg·L^{-1} CTC decreased TN removal efficiency from 77.4% to 64.1% and 53.4%, respectively. Meanwhile, the presence of 10 mg·L^{-1} CTC decreased the SOP removal efficiency from 96.3% to 78.1%. Mechanism studies indicated that CTC could affect the activities of reductase and the transformations of polyhydroxyalkanoates and glycogen, resulting in inhibition of denitrification and phosphorus uptake, which may be the major reason for the high level of CTC showing adverse influence on wastewater biological nutrient removal.

1. Introduction

Nowadays the occurrence, fate and toxicity of antibiotics have gained widespread attention of researchers around the world (Zhou et al., 2017). Over the past few decades, antibiotics were widely consumed to promote animal growth in aquaculture and prevent or treat animal and human diseases (Xiong et al., 2018). Although the antibiotic concentrations in wastewater treatment plants (WWTPs) are still at a subinhibitory level of μg/L in wastewater and mg/kg in sludge, it is believed to be a threat to biological wastewater treatment process because industrial wastewater containing antibiotics, such as pharmaceutical

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wastewater, is usually treated by biological process and its combination with other methods (e.g. advanced oxidation processes), and there is an increasing concern that antibiotic exposure may have long-term consequences (Liu et al., 2017).

Tetracyclines, including tetracycline, oxytetracycline and chlortetracycline, are one of the most commonly used antibiotics in human therapy and animal husbandry (Boxall et al., 2004). The United States consumes 9000 tons of antibiotics per year, of which 3000 tons are tetracyclines (Arikan et al., 2007). Chlortetracycline (CTC), as a broad-spectrum antibiotic in the tetracycline family, is widely applicated as feed additive for poultry and livestock leading to a serious environment pollution (Hamscher et al., 2002; Taheran et al., 2017). Once inside the cells, CTC can inhibit the sterol synthesis in cell membrane, which affects the permeability of cell membrane and further inhibits the growth of ether gram-positive bacteria or gram-negative bacteria (Bowman et al., 2011; Pulicharla et al., 2015).

Biological nutrient (nitrogen, phosphorus and organics) removal from wastewater has been proved to be an effective technique to avoid eutrophication problem (Li et al., 2014a). Biological nutrient removal includes many biochemical processes, such as anaerobic release and aerobic uptake of phosphorus, nitrification and denitrification. Biological phosphorus removal is achieved by polyphosphate-accumulating organisms (PAOs) through storing phosphorus within their cells as polyphosphate under aerobic environment (Chen et al., 2004). While nitrogen removal is accomplished by exposing nitrifers to aerobic environment for nitrification and exposing denitrifiers to anoxic condition for denitrification (Lee et al., 2010). Furthermore, the performance of these processes is closely related to the specific activities of some key metabolic enzymes including exopolyphosphatase (PPX), polyphosphate kinase (PPK), ammonia monooxygenase (AMO), nitrate reductase (NR), nitrite reductase (NIR), and nitrite oxidoreductase (NOR). These related microorganisms and key enzymes in biological nitrogen and phosphorus removal are susceptible to antibiotics (Chen et al., 2015; Pulicharla et al., 2015). Consequently, the presence of CTC may have a great impact on wastewater biological nutrient removal.

The inhibitory effects of antibiotics on biological nitrogen and phosphorus removal process have been reported abundantly (Collado et al., 2013; Katipoglu-Yazan et al., 2015; Meng et al., 2015). A significantly inhibitory influence of tetracycline and erythromycin antibiotics on nitrifier growth, the specific nitrification and nitratation rate at concentrations higher than 20 $mg \cdot L^{-1}$ was revealed (Alighardashi et al., 2009; Katipoglu-Yazan et al., 2015). While it was also reported that erythromycin showed promoting effect on the nitrification rate (Louvet et al., 2010). In addition, it had been found that new species emerged in the bioreactor bacterial community after being exposed to sulfamethoxazole dosage. In the meantime, *Betaproteobacteria* and *Gammaproteobacteria* became the dominant species due to the tolerance to the antibiotic (Collado et al., 2013).

Previous studies focused the impacts of antibiotics on bacterial community in activated sludge but ignored the inhibition on enzyme activity and the transformation of metabolic pathways related to biological nutrient removal. Consequently, the mechanism of the antibiotic toxicity on denitrification and phosphorus removal is still unclear. And surprisingly, the effect of CTC on biological nutrient removal process is rarely reported. The aim of this study was to investigate the long-term effect of CTC on biological nutrient removal considering bacteria viability, metabolic enzyme activities, and intermediate metabolites transformations including polyhydroxyalkanoates (PHAs) and glycogen to biological nutrient removal. Besides, possible mechanism for the influence of CTC on biological nutrient removal was also explored to comprehensively understand the impact of antibiotics on biological nutrient removal from wastewater.

2. Materials and methods

2.1. Synthetic wastewater

Synthetic wastewater containing 10 $mg \cdot L^{-1}$ PO_4^{3-}-P, 30 $mg \cdot L^{-1}$ NH_4^+-N and 300 $mg \cdot L^{-1}$ COD was prepared for experiment. The synthetic wastewater consisted of (per liter water): 0.3846 g CH_3COONa, 0.1144 g NH_4Cl, 0.0146 g KH_2PO_4 and 0.049 g $K_2HPO_4 \cdot 3H_2O$, 0.01 g $MgSO_4 \cdot 7H_2O$, 0.005 g $CaCl_2$ and 0.5 mL trace element solution which contained (per liter water): 1.50 g $FeCl_3 \cdot 6H_2O$, 0.18 g KI, 0.15 g $CoCl_2 \cdot 6H_2O$, 0.15 g H_3BO_3, 0.12 g $MnCl_2 \cdot 4H_2O$, 0.12 g $ZnSO_4 \cdot 7H_2O$, 0.06 g $Na_2MoO_4 \cdot 2H_2O$, 0.03 g $CuSO_4 \cdot 5H_2O$, and 10 g ethylenediamine tetra-acetic acid.

2.2. Parent reactor operation

A 36 L lab-scale anaerobic/oxic/anoxic parent sequencing batch reactor (SBR) was operated at 22 ± 1 °C. The seed sludge was obtained from a municipal wastewater treatment plant in Xiangtan, China. Each cycle consisted of 90 min anaerobic, 120 min aerobic, 150 min anoxic phase, 60 min 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 above was pumped into the reactor. In the aerobic stage, air was provided into the reactor to maintain dissolved oxygen (DO) concentration in the reactor around 2.0 $mg \cdot L^{-1}$. A magnetic stirrer was used for mixing except for the settling, decanting, and idle phases. The hydraulic retention time and sludge retention time were respectively maintained at 11 h and 12 d. The influent pH value was adjusted to 7.2 by adding 1 mol NaOH or 1 mol HCl. Stable operation was achieved after 60 days and then the sludge was withdrawn for the following toxicity tests.

2.3. Exposure experiments

The acute exposure experiments were carried out in 4 SBRs each with a working volume of 3 L. The SBRs were respectively received wastewaters containing no CTC (the control), 0.1, 1 and 10 $mg \cdot L^{-1}$ of CTC after seeded with the activated sludge from the parent SBR. The operated conditions of the 4 SBRs were consistent with the parent SBR. CTC (99% purity) was purchased from Shanghai Aladdin Biotechnology Co., Ltd., China.

Those above-mentioned SBRs containing different concentrations of CTC were operated for 120 days to investigate the potential chronic impact of CTC on biological nutrient removal. CTC was added with a certain amount every day to maintain CTC levels in the SBRs during the long-term operation.

2.4. Analytical methods

Samples were taken from the reactors during the experiments and were immediately filtered through a Whatman GF/C glass microfiber filter (1.2 mm). The filtrate was analyzed for soluble orthophosphate (SOP), ammonia (NH_4^+-N), nitrite (NO_2^--N) and nitrate (NO_3^--N), total nitrogen (TN), chemical oxygen demand (COD) and the filter was assayed for mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS), sludge volume index (SVI), glycogen, and PHAs. NH_4^+-N, NO_2^--N, NO_3^--N, SOP, COD, MLSS and MLVSS were measured using the standard methods (APHA, 1998). The glycogen and intracellular PHAs, including poly-3-hydroxybutyrate (PHB), poly-3-hydroxyvalerate (PHV) and poly-3-hydroxy-2-methylvalerate (PH2MV) were analyzed by the gas chromatography (Wang et al., 2009). The activities of PPX, PPK, AMO, NR, NOR and NIR were analyzed as to the reference (Louvet et al., 2010).

The release of lactate dehydrogenase (LDH) was measured to assay cell membrane integrity of activated sludge by using a commercially available kit (Roche Molecular Biochemicals). The cell viability was