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Effect of nitrite from nitritation on biological phosphorus removal in a sequencing batch reactor treating domestic wastewater

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ABSTRACT

Although nitrite effect on enhanced biological phosphorus removal (EBPR) has been previously studied, very limited research has been undertaken about the effect of nitrite accumulation caused by nitritation on EBPR. This paper focused on nitrite effect from nitritation on EBPR in a sequencing batch reactor treating domestic wastewater. Results showed that nitrite of below 10 mg/L did not inhibit P-uptake and release; whereas EBPR deterioration was observed when nitrite accumulation reached 20 mg/L. Due to P-uptake prior to nitritation, nitrite of 20 mg/L has no effect on aerobic P-uptake. The main reason leading to EBPR deterioration was the competition of carbon source. Batch tests were conducted to investigate nitrite effect on anaerobic P-release. Under sufficient carbon source, nitrite of 30 mg/L had no impact on poly-β-hydroxyalkanoate (PHA) storage; contrarily, under insufficient carbon source, denitrifiers competing for carbon source with phosphorus accumulating organisms resulted in decrease of PHA synthesis and P-release.

1. Introduction

Nitrogen removal via the nitrite pathway implies that the partial nitrification of ammonia to nitrite has been named nitritation, and the subsequent direct reduction of nitrite to N_2 gas, denitritation (Balmelle et al., 1992; Zhu et al., 2008). The application of nitritation–denitritation could lead to a considerable saving in aeration costs and external carbon sources as compared to the complete nitrification–denitrification (Hellinga et al., 1998; Jenicek et al., 2004; Fux et al., 2006; Zeng et al., 2010). The key to achieve nitritation–denitritation is the control of oxidation of ammonia to nitrite and the build-up of nitrite (Gao et al., 2009). As a result of nitritation, nitrite concentration often reached more than 20 mg/L, much higher than that in a conventional nitrogen removal system (Yang et al., 2007; Ma et al., 2009; Zeng et al., 2009). However, nitrite has been recognized as one inhibitor in microbial metabolism (Yarbrough et al., 1980). Previous studies have confirmed that high concentrations of nitrite inhibits microbial activities in biological wastewater treatment, such as inhibition on heterotrophic bacteria (Musvoto et al., 1999), nitrifying bacteria (Anthonisen et al., 1976; Vadivelu et al., 2006) and phosphate accumulation organisms (PAOs) (Zhou et al., 2007). Therefore, in the processes with nitrogen removal via the nitrite pathway, nitrite accumulation likely has an adverse impact on biological nutrient removal.

Enhanced biological phosphorus removal (EBPR) generally with simultaneous nitrogen removal is widely implemented in wastewater treatment plants (WWTP). PAOs can grow in EBPR process with alternating anaerobic and aerobic/anoxic conditions. Under anaerobic conditions, PAOs take up carbon source such as volatile fatty acids (VFAs) and store them in the form of poly-β-hydroxyalkanoates (PHA), using the energy generated from hydrolysis of polyphosphate (poly-P). Under aerobic or anoxic conditions, PAOs are able to take up excess phosphorus to form intracellular poly-P by using stored PHA as the energy source. The net removal of P can be achieved through wasting activated sludge when rich in poly-P (Oehmen et al., 2007).

Previous studies demonstrated that a certain amount of nitrite could inhibit anoxic/aerobic P-uptake of PAOs, even leading to deterioration of biological P removal (Meinhold et al., 1999; Saito et al., 2004; Kuba et al., 1996; Ahn et al., 2001; Hu et al., 2003; Sin et al., 2008; Yoshida et al., 2009). Using an anaerobic/aerobic/anoxic/aerobic SBR treating municipal wastewater, Yoshida et al. (2006) observed that nitrite exposure could inhibit aerobic phosphate uptake of PAOs and suggested that nitrite is one of the factors affecting stability of EBPR. Presently, the protonated species of nitrite, free nitrous acid (FNA) rather than nitrite is likely the actual inhibitor on the P-uptake by PAOs (Zhou et al., 2007). Zhou et al. (2007) indicated that FNA inhibits anoxic P-uptake at the low levels of 1.0×10^{-3}–2.0×10^{-3} HNO_2-N/L. Saito et al. (2004) reported that 0.5×10^{-3} HNO_2-N/L causes a severe inhibition of aerobic P-uptake, and more than 1.5×10^{-3} HNO_2-N/L results in almost complete inhibition. Moreover, as denitrifying phosphorus

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removal has been observed in many EBPR systems, it has been experimentally demonstrated that PAOs acclimated to nitrite are capable of using nitrite as sole electron acceptor to perform denitrification and phosphorus removal without inhibition (Guisasola et al., 2009; Jiang et al., 2006; Vargas et al., 2011). It should be noted that most of the research focused on the effect of nitrite/FNA on the activity of PAOs and the inhibition levels varied in a large range at different operation modes and wastewater types. Moreover, most of these studies were undertaken with addition of nitrite at the beginning of the aerobic or anoxic phase to investigate the impact of nitrite/FNA on biological P removal. Very limited research has been conducted about the effect of nitrite accumulation caused by nitritation–denitritation on EBPR. Therefore, the impact of nitrite accumulation on biological nutrients removal in a nitritation–denitritation system should be investigated further.

This study aims to (1) find an effective strategy to achieve shortcut nitrification and investigate the effect of nitrite accumulation on EBPR in an anaerobic/aerobic sequencing batch reactor (SBR) treating real domestic wastewater, (2) experimentally analyze the effect of nitrite accumulation on anaerobic P-release and aerobic P-uptake, and (3) investigate a controlling method to alleviate the nitrite inhibition, and maintain a stable EBPR performance.

2. Methods

2.1. Reactor and operation

Two lab-scale sequencing batch reactors (SBRs) fed with real domestic wastewater (composition given below) were used to carry out the experiments. SBR1 with a working volume of 11 L was operated for 180 days under anaerobic–aerobic conditions for anaerobic P-release and aerobic P-uptake. Even though in some instances the presence of nitrite in the anaerobic period makes it an actually anoxic period, we still defined this as anaerobic period to make the context consistent. Throughout the operational period, DO concentration was controlled at 0.5–1.0 mg/L, and each cycle included 2 h anaerobic duration, 1 h settling and 1 min decanting with 5.5 L supernatant discharged. To achieve partial nitrification to nitrite, aerobic duration was controlled at 3 h (day 1–30), 4 h (day 31–65) and 5 h (day 66–180), respectively. Therefore, cycle time was varying, 361 min (day 1–30), 421 min (day 31–65) and 481 min (day 66–180), respectively. The sludge retention time (SRT) in SBR1 was 20 days.

SBR2 with a working volume of 7 L was operated under anaerobic–aerobic conditions. Each cycle of 7 h consisted of 2 h anaerobic period and 4 h aerobic period, followed by 1 h settling and 1 min decanting with 3.5 L supernatant removed. The SRT in SBR2 was 8 days.

2.2. Wastewater and seed sludge

Both domestic wastewater and synthetic wastewater were used in this research. Domestic wastewater from a campus sewer line was pumped into a storing tank for sedimentation, and then fed into the SBR1 and SBR2. The influent characteristics are shown in Table 1.

Table 1
Characteristics of the domestic wastewater.

Contents (mg/L)	Average
COD	195 ± 28
NH_4^+-N	69 ± 10
TN	72 ± 11
PO_4^{3-}-P	6.3 ± 2.0

Synthetic wastewater was used in batch experiments containing per liter: 0.3–1.2 g NaAc, 180 mg $MgSO_4{\cdot}7H_2O$, 21 mg $CaCl_2{\cdot}2H_2O$, 3 mg peptone and 0.6 ml nutrient solution. The nutrient solution contained as shown in Smolders et al. (1994), per liter: 1.5 g $FeCl_3{\cdot}6H_2O$, 0.15 g H_3BO_3, 0.03 g $CuSO_4{\cdot}5H_2O$, 0.18 g KI, 0.12 g $MnCl_2{\cdot}4H_2O$, 0.06 g $Na_2MoO_4{\cdot}2H_2O$, 0.12 g $ZnSO_4{\cdot}7H_2O$, 0.15 g $CoCl_2{\cdot}6H_2O$ and 10 g ethylenediamine tetra-acetic acid (EDTA). When investigating the effect of nitrite accumulation on anaerobic metabolism of PAOs under insufficient carbon source, COD concentration in the synthetic wastewater was controlled at 200 mg/L. When investigating the effect of nitrite accumulation under sufficient carbon source, COD concentration was controlled at 800 mg/L.

The seed sludge was taken from the recycling sludge of Gao bei dian wastewater treatment plant in Beijing, which utilizes a typical anaerobic–anoxic–aerobic (A^2O) process to treat municipal wastewater and performs biological nutrients removal well. The wastewater composition was similar to that used in this study. After sludge acclimation for one month, a stable performance was achieved and the experiments begun.

2.3. Batch experiments

Tested sludge was taken from SBR2 at the end of aerobic stage. It was washed to remove nitrite and then divided into four parts. Each part of the sludge was put into a 1.5 L batch reactor. Four batch reactors were supplied with synthetic wastewater. The initial NO_2^--N concentration in four batch reactors was controlled at 0, 10, 20 and 30 mg N/L, respectively, by adding different amount of sodium nitrite to investigate the effect of nitrite on anaerobic phosphorus release. During the tests, pH was on-line controlled at 7.5 ± 0.05 by adding 0.5 M HCl or 0.5 M NaOH, and temperature was controlled at 25 ± 0.5 °C.

2.4. Analytical methods

NH_4^+-N, NO_2^--N, PO_4^{3-}-P, mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) were measured according to APHA standard methods (1998). DO and pH were measured on-line using DO/pH meters (WTW Multi 340i, Germany). Volatile fatty acids (VFAs) were measured using Agilent 6890N gas chromatography (GC) with an Agilent DB-WAXetr column (30 m × 1.0 μm × 0.53 mm) equipped, and the injection port and the flame ionization detector (FID) were operated at 220 °C and 250 °C. Temperature program was used: maintained at 80 °C for 1 min; reached up to 160 °C by 20 °C/min and then held for 1 min; increased by 5 °C/min to 180 °C and then held for 1 min. Analysis of PHA, consisting of poly-β-hydroxybutyrate (PHB) and poly-β-hydroxyvalerate (PHV), was performed using Agilent 6890N GC with an Agilent DB-1 column (30 m × 1.0 μm 0.53 mm). Weighed freeze-dried biomass, 2 ml chloroform and 2 ml methanol acidified with 3% H_2SO_4 were added into glass tubes, respectively, and then the tubes were heated in 100 °C for 20 h after being mixed. One milliliter of Milli-Q water was put into the tubes and mixed after cooling. After centrifugation, 1400 mL of the bottom organic phases was put into 2 mL tube, and 600 mL Milli-Q water was added and mixed. After centrifugation, 1 mL of the bottom organic phases was added into GC vial for analysis. The temperature of injector and FID detector were maintained at 200 °C and 250 °C. The temperature program was set as the following: held at 80 °C for 2 min; increased to 140 °C at the rate of 10 °C/min, and then maintained for 1 min.The concentration of free nitrous acid (FNA, HNO_2-N/L) was calculated as the formula (1) (Anthonisen et al., 1976):