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Inhibition of free ammonia to the granule-based enhanced biological phosphorus removal system and the recoverability

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HIGHLIGHTS

- The toxic threshold of FA concentration in P metabolism was 17.76 mg N L^{-1}.
- Acclimation took place by PAOs for FA concentration of 8.88 mg N L^{-1}.
- FA deteriorated the settleability, stability and integrity of the granules.
- FA could repress the excretion of extracellular polysaccharides.
- FA inhibition may provide a competitive advantage to GAOs over PAOs.

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ABSTRACT

The inhibition of free ammonia (FA) to the granule-based enhanced biological phosphorus removal (EBPR) system and the recoverability from macro- to micro-scale were investigated in this study. FA was found to seriously deteriorate the EBPR performance and sludge characteristic (settleability and morphology). The FA inhibitory threshold of 17.76 mg N L^{-1} was established. Acclimation phenomenon took place when poly-phosphate accumulating organisms (PAOs) were exposed for long time to constant FA concentration (8.88 mg N L^{-1}). The repressed polysaccharides excretion could lead to breaking the stability and integrity of the granules. Therefore, the reduced particle size and granule disintegration were observed. The molecular analysis revealed that FA had a significant influence on the microbial communities and FA inhibition may provide a competitive advantage to glycogen accumulating organisms (GAOs) over PAOs. Interestingly, the community composition was found irreversible by recovery (Dice coefficients, 36.3%), although good EBPR performance was re-achieved.

1. Introduction

Excessive phosphorus loading to natural waters has been known for decades to accelerate eutrophication and leads to decline in water quality. The enhanced biological phosphorus removal (EBPR) process is a preferable alternative to the chemical precipitation of phosphorus in wastewater treatment (Schönborn et al., 2001). EBPR has been used in numerous full-scale plants for many years. Granules are the form of self-immobilization of microbial flocs under certain conditions (such as selective pressure and hydrodynamic shear force) in the wastewater treatment process. In contrast to the conventional activated sludge, biogranules have a regular, dense, strong structure and good settling properties. They enable high biomass retention and withstand high-strength wastewater and shock loadings (Liu and Tay, 2004). Thus, granular sludge reactors are desirable in wastewater treatment processes. Therefore combining EBPR with the granule process has resulted in the formation of poly-phosphate accumulating granules. These granules represent innovative and promising strategies in the biological wastewater treatment and attract increasing interests (Zhang et al., 2011; Wu et al., 2012; Zheng et al., 2013).

The EBPR process is indeed capable of efficient phosphorus removal, while sudden breakdown of EBPR under full-scale conditions has been observed in many cases. One reason of the disturbances is the addition of sudden shock loadings that arise from the periodic discharge of industrial effluents with high concentrations of COD and/or nutrients (Broughton et al., 2008). Shock loadings can not only result in temporary process upsets, but also lead to the deterioration of process performance through affecting the biomass composition of the sludge (Freitas et al., 2009). In the last decades, the treatment of high ammoniacal concentration effluents has become great interest. Various effluents can contain several hundred milligrams of nitrogen per liter (such as the supernatants from anaerobic digestion, leachates from municipal landfill). When such effluents are discharged into the environment,

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depletion of receiving-water oxygen resources and eutrophication can inevitably occur, which shows the importance of high ammonia treatment. The treatment of nitrogen is carried out generally biologically (Pambrun et al., 2008). In practice, nitrification, denitrification and EBPR process often take place simultaneously in a single reactor unit. Recently, free ammonia (FA) rather than ionic ammonium has been commonly thought to have a broad inhibitory effect on bacterial metabolism (Vadivelu et al., 2006, 2007).

The inhibition of FA on ammonia and nitrite oxidizing bacteria (AOB and NOB) activities has been studied by many researchers over the past few decades. Anthonisen et al. (1976) reported that FA initiated inhibition on *Nitrobacter* at 0.08–0.82 mg NH_3–N L^{-1}, while the threshold value for *Nitrosomonas* was 8.2–123.5 mg NH_3–N L^{-1}. Vadivelu et al., (2006) found that FA of up to 16.0 mg NH_3–N L^{-1} did not have any inhibitory effect on either the catabolic or anabolic processes of the *Nitrosomonas* culture. However, *Nitrobacter* likely ceased to grow at an FA level of above 6 mg NH_3–N L^{-1}, as reported by Vadivelu et al., (2007). So NOB has been proved to be much more sensitive to FA than AOB. Additionally, with the wide application of granular sludge in biological wastewater treatment, Yang et al. (2004) investigated the inhibition of FA to the development of aerobic granules for the first time. It was found that aerobic granules formed only when the FA concentration was less than 23.5 mg L^{-1} and nitrification was completely inhibited at a FA concentration greater than 10 mg L^{-1}.

To date, almost all the work has focused on the FA toxicity threshold, inhibitory effect on the organisms' metabolism and the toxic mechanism in systems of nitrogen removal. However, none of the research looks into the impact of FA on the granular sludge especially the PAOs granules from macro- to micro-scale and the recovery process. Actually, the urban sewage in China is a mixture of domestic and industrial wastewater containing high levels of nitrogen and phosphorus but limited carbon source (Sun et al., 2011). In recent years, with the wide application of granular sludge in biological wastewater treatment, it is worth carrying on the fundamental research to understand the impact of FA on granular sludge and then improve the treatment process. Therefore, the main objective of this work is to investigate the effect of FA on the granule-based EBPR system from macro- to micro-scale and the recoverability at different FA levels. In order to characterize the microbial community structure in bioreactors, both polymerase chain reaction–denaturing gradient gel electrophoresis (PCR–DGGE) and fluorescence in situ hybridization (FISH) were employed. Then the recovery experiment was conducted after EBPR performance being completely deteriorated to verify if the inhibition was reversible. Meanwhile, the recoverability of microbial community structure was also analyzed.

2. Methods

2.1. Cultivation of EBPR population and granules

Two lab-scale (working volume was 10 L) anaerobic–aerobic sequencing batch reactors (SBR) were used for the enrichment of PAOs. According to Lu et al. (2006), the sole carbon source in the synthetic feed was alternated between acetate and propionate with a switching frequency of 10 days, in order to provide further selective advantages to PAOs over GAOs. Details of the reactor design, operation and performance can be found in Zheng et al. (2013). The EBPR biomass was acclimated for more than 60 days to obtain high enrichment of PAOs for the granulation. PAOs were accounting for 84.3 ± 4.2% of all bacteria as assessed by FISH quantification. For three months of operation, the granule-based EBPR system showed steady phosphorus removal performance and mature granular sludge was obtained. The granular sludge concentration (mixed liquor suspended solids, MLSS) was about 2500 mg L^{-1}.

2.2. Synthetic medium

Acetate and propionate were used as the mixed carbon source in the ratio of 1/3 according to their chemical oxygen demand (COD). K_2HPO_4 and KH_2PO_4 were used to provide P source in the synthetic wastewater. At the beginning of the anaerobic stage of each cycle, they were added into each batch reactor to reach initial COD concentration of 200 mg L^{-1} and PO_4^{3-}–P concentration of 10 mg L^{-1}. The compositions of the synthetic wastewater included (per liter): 0.256 g CH_3COONa, 0.4 mL CH_3CH_2COOH, 0.0875 g KH_2PO_4, 0.147 g $K_2HPO_4{\cdot}3H_2O$, 0.1845 g $MgSO_4{\cdot}7H_2O$, 0.0222 g $CaCl_2$, 0.0015 g peptone, 0.0015 g yeast extract powder, 0.0012 g allylthiourea (ATU) and 0.6 mL trace elements solution. Moreover, an ammonium stock solution (ammonium chloride at a concentration of 12 g NH_4^+–N L^{-1}) was added to the batch reactors in different volumes at the beginning of each experiment, which resulted in initial concentrations of ammonium varying between 15 and 600 mg NH_4^+–N L^{-1}, as summarized in Table 1. FA can be determined through the ammonium concentration (NH_4^+–N, mg L^{-1}), pH and temperature (T, °C) by the formula $\{1.214 \times [NH_4^+\text{–N}] \times 10^{pH}\}/\{\exp[6344/(273+T)] + 10^{pH}\}$ reported by Anthonisen et al. (1976). ATU was supplied in the feed to inhibit nitrification. As such, the consumption of ammonium could be fully attributed to bacterial growth. The composition of the trace elements solution was the same as that described by Smolders et al. (1994).

2.3. Batch tests

Four parallel SBR reactors (termed as R1, R2, R3 and R4) with the working volume of 10 L were used in this study. The cultivated granules were taken as the seed sludge for R1–R4. The reactors had a 6 h cycle time with the following sequence: 5 min feeding of 2.5 L synthetic wastewater, 2.5 h of anaerobic phase, 3 h of aerobic phase, 5 min of sedimentation, 5 min for the extraction of 2.5 L of supernatant and 15 min idling. There were 4 cycles per day and the volume exchange ratio was 1/4. The solids retention time (SRT) was kept at 8 days controlled by biomass wasting at the end of the aerobic phase. The reactor covered with a lid of plexiglass in the anaerobic period of every cycle happen manually so that the air was excluded. The agitation speed during the operation was controlled at 300 rpm to make sure the complete mixing of the nutrient. Dissolved oxygen (DO) was controlled at 6–7 mg L^{-1} during the aerobic phase. The pH was adjusted to 7.5–8.0 with 0.5 M NaOH and 0.5 M HCl during the operation.

The experiment was divided into two phases: inhibition and recovery (Table 1). R1 was the control group and served as a reference. R2, R3 and R4 were the test groups and operated at gradually step-increased concentrations of ammonium in the range of 20–600 mg NH_4^+–N L^{-1} in order to find the threshold value of this

Table 1
Experiment scheme.

Cultivation phase		Ammonium concentrations after feed (mg NH_4^+–N L^{-1})			
		R1 (Control)	R2	R3	R4
Inhibition	Phase 1 (day 1–4)	15	20	30	40
	Phase 2 (day 5–8)		60	80	100
	Phase 3 (day 9–29)		200[a]	400[b]	600[c]
Recovery	Phase 4 (day 30–51)		15		

[a] The respective FA concentration in R2 was calculated as 8.88 mg N L^{-1}
[b] The respective FA concentration in R3 was calculated as 17.76 mg N L^{-1}
[c] The respective FA concentration in R4 was calculated as 26.64 mg N L^{-1}