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The long-term effect of nitrite on the granule-based enhanced biological phosphorus removal system and the reversibility

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HIGHLIGHTS

- Nitrite deteriorated the settleability, stability and integrity of the granules.
- Nitrite could inhibit the excretion of polysaccharides.
- Granules with lower proteins/polysaccharides had better structure and function.
- The community composition was not reversible by recovery.
- GAOs had stronger resistibility and higher recovery rate than PAOs.

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ABSTRACT

This study investigated the long-term effect of nitrite on the granule-based enhanced biological phosphorus removal (EBPR) system and the reversibility from macro- to micro-scale. Nitrite was found to seriously deteriorate the EBPR performance and result in severe sludge bulking. The inhibited polysaccharides excretion could lead to breaking the stability and integrity of the granules. Therefore, the reduced particle size and granule disintegration were observed. In this study, granules with lower ratio of proteins to polysaccharides (1.76) had better structure and function than the higher (3.84). Experimental results demonstrated that the microbial community structure was largely changed due to the presence of nitrite. In comparison, glycogen accumulating organisms (GAOs) had stronger resistibility and higher recovery rate than poly-phosphate accumulating organisms (PAOs). Interestingly, the community composition was unable to recover (Dice coefficients, 33.0%), although good EBPR performance was achieved only by propagating other types of PAOs.

1. Introduction

Enhanced biological phosphorus removal (EBPR) process has been recognized as the most suitable way to avoid the eutrophication problem because phosphorus acts as a limiting nutrient for algal blooms (Ahn et al., 2001). It 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 the selective pressure) in the wastewater treatment process. Microbial granules play an important role in biological wastewater treatment due to their advantages over the conventional sludge flocs, such as a denser and stronger aggregate structure, better settle ability and solid–liquid separation, higher biomass concentration, greater ability to withstand shock loadings and toxicity. So the granulation of EBPR results in the formation of poly-phosphate accumulating granules which represent innovative and promising strategies in the biological wastewater treatment and attract increasing interests (Zhang et al., 2011; Wu et al., 2012).

The EBPR process is indeed capable of efficient phosphorus removal, while disturbances and prolonged periods of insufficient P removal have been observed at full-scale plants on numerous occasions under conditions that are seemingly favorable for EBPR (Oehmen et al., 2007). Its successful operation depends on numerous process operational factors, particularly the variation in wastewater quality (Wang et al., 2011a). In practice, EBPR is often combined with biological nitrogen removal and nitrite is an intermediate of the biological oxidation from ammonia to nitrate. Recently, nitrite has been commonly thought to have a broad inhibitory effect on bacterial metabolism. Free nitrous acid (FNA), the protonated form

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of nitrite, was considered the true inhibitor on bacteria rather than the nitrite anion itself (Zhou et al., 2008, 2011; Pijuan et al., 2010). FNA can be determined through the nitrite concentration, pH and temperature by the formula reported by Anthonisen et al. (1976).

Recently, effect of nitrite and FNA on biological nitrogen and phosphorus removal system has been studied widely and intensively. Saito et al. (2004) found that 2 mg N L^{-1} of nitrite caused already a severe inhibition of aerobic phosphate uptake and more than 6 mg N L^{-1} of nitrite resulted in almost complete inhibition. FNA also could strongly inhibited both anabolic (growth, P-uptake and glycogen production) and catabolic (poly-hydroxyalkanoates oxidation) processes of the *Accumulibacter* PAOs to different extents. And FNA may disadvantage PAOs in their competition with GAOs in EBPR systems (Pijuan et al., 2010). It has been well known that PAOs and GAOs are closely related bacteria in metabolism. GAOs are also capable of anaerobic VFAs uptake but without contributing to phosphorus removal. Moreover, Zhou et al. (2008) investigated the nitrite inhibitory effect under various pH conditions using denitrifying EBPR sludge. The inhibition was found to be reversible, and the recovery rate was independent of the inhibition duration, but dependent on the FNA concentration the biomass was exposed to. Both of Weon et al. (2002) and Zhou et al. (2011) suggested that the mechanism of nitrite toxicity was associated with its effect on bacterial membranes and energy generation. Furthermore, nitrite and FNA accumulation were known to inhibit phosphorus uptake which may be involved in damage to polyphosphate kinases (PPK) or poly-hydroxyalkanoates (PHAs) oxidation which was reported by Wang et al. (2011b). Additionally, Saito et al. (2004) described that nitrite had negative effect on the enzyme system relating to phosphate uptake and poly-phosphate formation. However, Zeng et al. (2011) reported that enough supply of carbon source could effectively alleviate the adverse impact of nitrite accumulation on EBPR.

So far most of the experimental studies focused on the nitrite or FNA toxicity threshold, inhibitory effect on the organisms' metabolism and the mechanism with short-term or cycle tests. However, the long-term effect of nitrite on the granular sludge especially the PAOs granules from macro- to micro-scale and the recoverability have not been well investigated. Actually, the urban sewage in China as a mixture of domestic and industrial wastewater is containing high nitrogen and phosphorus but with limited carbon source. Different environmental conditions during long-term operation would result in the selection of different microbial populations. In recent years, with the wide application of granular sludge in biological wastewater treatment, it is essential to carry on this work. Therefore, the long-term effect of nitrite on the granule-based EBPR system including the sludge characteristics, the size and morphology of the granules, extracellular polymeric substances (EPS), phosphorus release and uptake rate, PAOs/GAOs competition and the change of microbial community structure were investigated in this study. In order to characterize the microbial community structure in bioreactors, both PCR-DGGE (polymerase chain reaction-denaturing gradient gel electrophoresis) and fluorescent 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

The biomass of PAOs was enriched using a lab-scale anaerobic–aerobic SBR. Details of the reactor design, operation and performance can be found in Fang et al. (2012). Therefore, high enrichment of *Candidatus* "Accummulibacter phosphatis" was obtained for the granulation. PAOs were accounting for 80% of all bacteria as assessed by fluorescence in situ hybridization (FISH) quantification. For 3 months of operation, the granule-based EBPR system showed steady phosphorus removal performance and mature granular sludge was obtained. The granular sludge concentration was about 2500 mg L^{-1}.

2.2. Synthetic medium

In this study, acetate and propionate in the ratio of 1/3 were used as the mixed carbon source. NH_4Cl, K_2HPO_4 and KH_2PO_4 were used to provide the N and P sources in the synthetic wastewater. At the beginning of the anaerobic stage of each cycle, they were quickly added into each batch reactor to reach initial COD concentration of 200 mg L^{-1}, NH_4^+-N concentration of 15 mg L^{-1} and PO_4^{3-}-P concentration of 10 mg L^{-1}. The compositions of the synthetic wastewater were including (per liter water): 0.256 g CH_3COONa, 0.4 mL CH_3CH_2COOH, 0.0875 g KH_2PO_4, 0.147 g $K_2HPO_4{\cdot}3H_2O$, 0.2293 g NH_4Cl, 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), 0.5112 g $NaClO_3$ and 0.6 mL trace elements solution. ATU was supplied to inhibit nitrification and $NaClO_3$ was a selective inhibitor of NO_2^--N oxidation (Liu et al., 2011). The removal of NH_4^+-N in the aerobic phase was only used for microbial cell synthesis. The composition of the trace elements solution was the same as the description of Smolders et al. (1994). Moreover, a required amount of sodium nitrite was added to the influent from a concentrated solution resulting in a series of concentrations described below.

2.3. Batch tests

Three parallel SBR reactors (termed as R1, R2 and R3) with the working volume of 10 L were used in this study. The cultivated granules were taken as the seed sludge of R1–R3. 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 four cycles per day and the volume exchange ratio was 1/4. The solids retention time (SRT) was kept at 7 days controlled by biomass wasting at the end of the aerobic phase. In the anaerobic period, the reactors were covered so that the air was excluded. The agitation speed during the operation was controlled at 300 rpm using the stirrer to make sure the nutrient deserve complete mix. Dissolved oxygen (DO) was controlled at 6–7 mg L^{-1} during the aerobic phase. The pH was adjusted to about 7.0 with 0.5 M NaOH and 0.5 M HCl during the operation.

The experiment was divided into three phases. In the first 14 days, the initial nitrite concentrations were 10, 20 and 30 mg L^{-1} for R1, R2 and R3, respectively. Subsequently the nitrite loading was reduced in half for 8 days to investigate if the serious inhibition would be relieved. Therefore, phase 1 was defined as the full loading and phase 2 as the half loading. After 22 days completely being destroyed which was represented in the deterioration of dephosphorization ability, the recovery experiments with no nitrite addition were performed in order to test for the reversibility of this inhibition. The sludge in each batch reactor was washed with distilled water for three times to remove the residual nitrite on day 23. The recovery tests lasted for 22 days until the efficiency of phosphorus removal was improved and reached to a steady state for keeping 10 days at least.

2.4. Analytical methods

Samples were taken from each treatment at the beginning of batch tests, the end of anaerobic stage and the end of aerobic stage,