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Phosphorus metabolism and population dynamics in a biological phosphate-removal system with simultaneous anaerobic phosphate stripping

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HIGHLIGHTS

- Intracellular poly-P is an important factor affecting biological phosphorus removal.
- A shift from PAM to GAM occurred when poly-P accumulation decreased.
- The abundance of GAOs increased with the decreasing poly-P content.
- The microbial community structure changed a lot with decreasing poly-P content.

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ABSTRACT

In this study, the metabolism of phosphorus and changes in population dynamics were investigated via simultaneous chemical stripping in sidestream in an acetate-fed sequencing batch reactor. The synthesized intracellular polyphosphate (poly-P) by polyphosphate-accumulating organisms (PAOs) gradually decreased when the biomass was subjected to 83 d of P stripping. Initially, the P removal efficiency of the system improved from 94.3% to 96.9%. Thereafter, a relatively high level of P in effluent was observed, during which time the stoichiometric ratios of $P_{release}/HAc_{uptake}$ decreased, $Glycogen_{degraded}/HAc_{uptake}$ and poly-β-hydroxyvalerate/PHA increased. The results revealed that a metabolic shift from polyphosphate-accumulating metabolism to glycogen-accumulating metabolism. Correspondingly, PAOs declined to less than 1% of the population, glycogen-accumulating organisms proliferated to almost 20% instead. The results of PCR–DGGE also indicated that the microbial community structure considerably changed in response to the gradually decreasing poly-P content. These findings imply that intracellular poly-P level is important for the stable of P removal system. Furthermore, it suggests that it is not a stable and effective way for P recycling from anaerobic stage of the biological P removal system in sidestream.

1. Introduction

Biological processes, known as enhanced biological phosphorus removal (EBPR), are more economical and environmentally sound alternatives to the chemical treatment schemes in municipal wastewater treatment systems. However, given that numerous factors are known to influence EBPR (Song et al., 2009; Ersu et al., 2010; Bassin et al., 2012), problems with the stability of EBPR systems have been highlighted. Therefore, the operational unpredictability of EBPR has necessitated supplementary chemical precipitation of phosphate (Gebremariam et al., 2011). In particular, an increasing number of stringent discharge requirements limit the release of P in effluents from municipal wastewater treatment plants in China.

The use of simultaneous chemical addition results in the suppression on biological P removal, and the competition between chemical and biological P removal was discussed extensively in the review of de Haas et al. (2000). As an alternative, the Phostrip process, or a sidestream process, was designed. The Biologisch-Chemische-Fosfaat-Stikstofverwijdering (BCFS) process was subsequently developed for the efficient combination of chemical and biological P removal in which anaerobic supernatants were stripped from the biological system with chemical precipitate for P recovery. Biological P removal activity may be decreased if stripping flow rate was exceeded the optimal one (Hao and Loosdrecht, 2006). It was thought that the P removal in the anaerobic stage caused the decrease of P content in the form of polyphosphate

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(poly-P) in sludge, which would cause serious problems during the downstream sludge treatment (sludge digestion) (Barat and van Loosdrecht, 2006). Therefore, the influence of simultaneous anaerobic phosphate stripping on biological phosphorus removal cannot be neglected. But how chemical phosphorus stripping resulting deterioration of biological phosphorus removal is still unclear.

According to Brdjanovic et al. (1998), acetate was not taken up and polyphosphate-accumulating organisms (PAOs) could not use glycogen conversion to poly-hydroxyalkanoate (PHA) as the sole energy under conditions of poly-P limitation resulting in glycogen surplus in the biomass. But a previous study (Liu et al., 1994) suggested that glycogen, instead of poly-P, is an energy source for acetate uptake when EBPR activity deteriorates. Zhou et al. (2008) showed that PAOs could absorb acetate and store it as PHA using glycogen as the primary energy source under poly-P limited conditions. A similarly result was reported by Erdal et al. (2008). Moreover, in the study of Acevedo et al. (2012), a shift from PAOs to glycogen-accumulating organisms (GAOs) metabolism occurred with the low level of poly-P storage and the effects of poly-P storage levels on the competition between different PAOs in EBPR system have been investigated using fluorescence in situ hybridization (FISH). In most cases, the poly-P content was minimised by reducing the phosphorus feeding concentration and/or decanting the supernatants of the anaerobic stage at short-term exposure. The changes in metabolism or population type may originate from a temporal adaptability to the changed environment. Overall, the investigations of biological P removal and microbial diversity under the conditions of decreasing poly-P content are not yet well established. The feasibility of phosphate recycling from the main reaction zone of the biological P removal system is also worth further study.

To get a better understanding of the impact of decreasing poly-P content on EBPR system, a portion of enriched P supernatants was stripped from the anaerobic stage of mainstream biological treatment by a long-term chemical precipitation in sidestream. In this study, both the performance of P removal and microbial community were investigated to assess the effectiveness of the EBPR process with decreasing poly-P content by combining chemical analysis and microbial community structure evolution based on FISH and polymerase chain reaction-denaturing gradient gel electrophoresis (PCR–DGGE). The potential and possibility of P recycling from anaerobic stage of the biological P removal system in sidestream was also discussed, based on the findings in this investigation.

2. Methods

2.1. Reactor setup and operation

The experiments were conducted in an anaerobic/oxic (AO)-SBR under a working volume of 10 L. The SBR was operated in cycles of 8 h, with each cycle consisting of 120 min anaerobic and 210 min aerobic reaction, followed by 135 min settling and 15 min decantation. Temperature was controlled at 20 ± 1 °C. 5 L of the filling volume in each cycle for the first 2 min of the anaerobic stage, which implied the hydraulic retention time (HRT) was 16 h. The solids retention time (SRT) was approximately 11 d by withdrawing the sludge from the reactor at the end of the aerobic stage.pH of the system which fluctuated between 7.1 and 8.3 was monitored online. Oxygen concentration in the aerobic phase was maintained at above 2 mg L^{-1}, was measured using HACH-HQ40d (USA). COD, orthophosphate (PO_4^{3-}), and pH were measured in samples taken from every 15 or 30 min of the cycle. The P content in the sludge (P%) was monitored every 3 d. Moreover, total phosphorus (TP), mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) were measured at the end of the aerobic stage. Most of the samples were repeatedly analysed.

Experiments were conducted in three operational stages: Stage I: A laboratory scale sequencing batch reactor was operated in a steady state, displaying excellent EBPR performance.

Stage II: This stage lasted for 83 d, and subjected to side-stream stripping treatment once every 3 cycles. At the end of the anaerobic stage, after a settling period, 5 L enriched phosphorus supernatants were separated from the SBR system into a chemical basin for chemical P precipitate. $FeCl_3{\cdot}6H_2O$ was used as a coagulant and coagulation/flocculation was controlled using a ferric to P molar ratio of 1.40, stirring intensity of 275 rpm for 30 s in coagulation, 60 rpm for 18 min in flocculation, and 20 min in precipitation. Then, the treated supernatants were returned to the aerobic stage. A schematic diagram of the experimental setup is depicted in Fig. SM-1 of Supporting Material (SM).

Stage III: Side-stream stripping operation was terminated and the same operating conditions as stage I. This stage lasted for 35 d to determine whether the AO process can replenish its P removal capacity without stripping.

2.2. Wastewater

The detailed compositions of synthetic wastewater were shown as the following in mg L^{-1} (except for nutrient solutions): 400 COD (CH_3COONa), 76.5 NH_4Cl, 44 KH_2PO_4, 180 $MgSO_4{\cdot}7H_2O$, 14 $CaCl_2{\cdot}2H_2O$, 0.5 mL $L^{-1}_{influent}$ nutrient solution. Acetate was selected as the sole carbon source because it was the most common VFAs in domestic wastewaters (Chen et al., 2004). The nutrient solutions consisted of following compounds in mg L^{-1}: 180 KI, 150 H_3BO_3, 120 $MnCl_2{\cdot}4H_2O$, 150 $CoCl_2{\cdot}6H_2O$, 60 $Na_2MoO_4{\cdot}2H_2O$, 30 $CuSO_4{\cdot}5H_2O$, 120 $ZnSO_4{\cdot}7H_2O$, 1.54 $FeSO_4{\cdot}7H_2O$, 10 EDTA.

2.3. Analytical work

Daily analyses of COD, MLSS, MLVSS, PO_4^{3-} and sludge volume index (SVI) were analysed according to the standard methods (APHA, 1998). TP was converted to orthophosphate through digestion with potassium persulfate; then, the method for orthophosphate determination was applied. The P% is calculated by (TP-P_e)/MLSS × 100, where P_e is the phosphate in the effluent in mg L^{-1}. Samples for the determination of dissolved iron were immediately filtered using 0.22 μm filter membrane and then acidification by hydrochloric acid (1 M) before conducted by atomic absorption spectroscopy (GBC Avanta PM-GF3000, Australia). Glycogen was measured by the anthrone method (Liu et al., 2007). PHA (Ballistreri et al., 2001) was analysed by the method proposed by gas chromatography method (Agilent 6890N, USA). The total PHAs was calculated as the sum of measured poly-β-hydroxybutyrate (PHB) and poly-β-hydroxyvalerate (PHV). The glycogen, MLSS and MLVSS concentrations are the averages of duplicate measurements. Average values are presented in the text with standard deviation.

Biomass was characterised using FISH techniques to quantify the amount of PAOs and GAOs present in the reactor according to Amann et al. (1990). The 16S rRNA-targeted oligonucleotide probes used in this study were FITC-labelled EUBMIX (equal amounts of EUB338-I, EUB338-II and EUB338-III) for the entire bacteria (Daims et al., 1999), Cy-3-labelled PAOMIX (equal amounts of PAO462, PAO651 and PAO846) to target PAOs (Crocetti et al., 2000) and Cy-5-labelled GAOMIX (equal amounts of GAO431, GAO989) for GAOs (Crocetti et al., 2002). When necessary, the sludge flocs were briefly dispersed to permit cell counting. The fixed cells were sonicated for 60 s with an ultrasonic processor (Vibracell, Sonics) at a pulse of 5 s and an output power of 4W. An inverted confocal laser scanning microscopy (CLSM, Leica TCS SP8,