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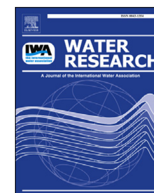

Calcium effect on the metabolic pathway of phosphorus accumulating organisms in enhanced biological phosphorus removal systems

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ABSTRACT

Phosphorus accumulating organisms (PAOs) have been found to act as glycogen-accumulating organisms (GAOs) under certain conditions, thus, the deterioration in the performance of enhanced biological phosphorus removal systems is not always attributed to the proliferation of GAOs. In this work, the effects of calcium on the metabolic pathway of PAOs were explored. It was found that when the influent Ca^{2+} concentration was elevated, the tendency and extent of extracellular calcium phosphate precipitation increased, and the intracellular inert Ca-bound polyphosphate was synthesized, while the microbial population remained almost unchanged. The changes in the ratios of phosphorus released/acetate uptaken, the glycogen degraded/acetate uptaken and the poly-β-hydroxyalkanoates synthesized/acetate uptaken during the anaerobic period confirm that, as the influent Ca^{2+} concentration was increased, the polyphosphate-accumulating metabolism was partially shifted to the glycogen-accumulating metabolism. At an influent Ca^{2+} around 50 mg/L, in addition to the extracellular calcium phosphate precipitation, the intracellular inert Ca-bound polyphosphate synthesis might also be involved in the metabolic change of PAOs. The results of the present work would be beneficial to better understand the biochemical metabolism of PAOs in enhanced biological phosphorus removal systems.

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1. Introduction

Phosphorus accumulating organisms (PAOs) and glycogen-accumulating organisms (GAOs) are the two most important types of microbial populations in enhanced biological phosphorus removal (EBPR) systems (Seviour et al., 2003; Oehmen et al., 2007). The deteriorated EBPR performance is usually linked to the proliferation of GAOs. The biochemical metabolism of PAOs is recognized as polyphosphate-accumulating metabolism (PAM) (Comeau et al., 1986; Mino et al., 1987; Pereira et al., 1996; Hesselmann et al., 2000; Yagci et al., 2003) and that of GAOs as glycogen-accumulating metabolism (GAM) (Satoh et al., 1994; Filipe et al., 2001a; Zeng et al., 2003; Acevedo et al., 2012). PAOs and GAOs seem to have similar metabolic pathways, while the main difference is that GAOs have no capacities for anaerobic polyphosphate (polyP) degradation and aerobic polyP synthesis (Seviour et al., 2003; Oehmen et al., 2007). Thus, for GAOs, the energy for the uptake of volatile fatty acids (VFAs) to synthesize poly-β-hydroxyalkanoates (PHAs) in anaerobic phase can be obtained from glycogen degradation only. Subsequently, the extra reducing equivalents (nicotinamide adenine dinucleotide, NADH) due to the greater degradation of glycogen will be consumed through the succinate–propionate pathway, leading to the more synthesis of polyhydroxyvalerate (PHV) compared with PAOs (Satoh et al., 1994; Filipe et al., 2001a; Zeng et al., 2003; Acevedo et al., 2012). Therefore, PAOs and GAOs have the different sources and consumptions of energy and reducing equivalent (Zhou et al., 2008; Acevedo et al., 2012).

PAOs may tune their metabolic pathway to partially use GAO metabolism under certain conditions (Erdal et al., 2008; Barat et al., 2006, 2008; Zhou et al., 2008; Acevedo et al., 2012; Welles et al., 2014). The metabolic pathway of PAOs could be shifted between the dominance of PAM and GAM along with the change of intracellular polyP level, which was confirmed by the evolution of PHA and glycogen in EBPR systems (Acevedo et al., 2012). Barat et al.

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(2006, 2008) found that the ratio of phosphorus released to acetate uptaken ($P_{release}/HAc_{uptake}$) declined as the influent Ca^{2+} concentration was increased, indicating the reduced availability of intracellular polyP. This implies the change of the metabolic pathway of PAOs. However, the mechanisms behind this process are not well understood yet, and no direct evidence about intracellular glycogen and PHA evolutions is available to validate the effects of Ca^{2+} on the shift of PAO metabolic pathway.

The Ca^{2+} concentration in municipal wastewater is usually in a range of 30–100 mg/L (Arvin and Kristensen, 1983; Carlsson et al., 1997). Calcium phosphate (Ca-phosphate) precipitation often occurs in EBPR systems, especially in biofilm or granular sludge systems (Arvin and Kristensen, 1983; Maurer et al., 1999; Barat et al., 2008; Mañas et al., 2011), which is considered to trigger a metabolic shift of PAOs (Barat et al., 2008). Besides, calcium, in addition to magnesium (Mg) and potassium (K), is also an important counter-ion of intracellular polyP granules (Schönborn et al., 2001). Thus, it was assumed that the intracellular polyP could be mainly divided into two types: the "active" Mg/K-bound polyP that was involved in the process of anaerobic degradation and aerobic synthesis, and the "inert" Ca-bound polyP, which remained stable under anaerobic conditions (Schönborn et al., 2001). The Ca-bound polyP synthesis has been speculated to affect the PAO metabolism, but has not been confirmed due to the lake of analysis of different types of intracellular polyP (Barat et al., 2006). Thus, it is not clear whether and how the two processes, i.e., the process of extracellular Ca-phosphate precipitation and the intracellular Ca-bound polyP synthesis, influence the metabolic pathways of PAOs at various Ca^{2+} concentrations yet, and this needs in-depth investigations.

In this work, the effects of calcium on the metabolic pathways of PAOs were examined. The kinetic process and speciation of extracellular Ca-phosphate precipitation, the intracellular inert Ca-bound polyP synthesis, and the microbial population dynamics at different influent Ca^{2+} concentrations were investigated. In addition, the ratio of phosphorus released/acetate uptaken, and the evolution of glycogen and PHA in the anaerobic phase were also explored to confirm the metabolic change of PAOs. On the basis of these results, the mechanisms behind the shift of PAO metabolic pathway at different Ca^{2+} concentrations were proposed to better describe the biochemical metabolism of PAOs in EBPR systems for the treatment of wastewater rich in Ca^{2+}.

2. Materials and methods

2.1. Operation of the EBPR granular sludge reactor

A laboratory-scale EBPR granular sludge sequencing batch reactor (SBR) with a working volume of 5.5 L (diameter of 10 cm and height/diameter ratio of 7) was operated at 20 ± 1 °C for 230 days. The granular sludge reactor was inoculated with activated sludge rich in PAOs, which was collected from an EBPR reactor reported previously (Zhang et al., 2013). pH was not controlled in the operation. Each cycle consisted of 120 min anaerobic period, 232 min aerobic period, 3 min settling and 5 min decant period. Sludge mixture of 270 mL was discharged each day at the end of the first aerobic phase. The hydraulic retention time and solids retention time (SRT) were set at 12 h and 20 d, respectively. Stirring was provided at the anaerobic stage. The air flow rate was 3.0 L/min for aeration. A synthetic wastewater with sodium acetate as the sole carbon source was fed to the reactor in the initial 10 min, which included (in mg/L): acetate, 200 in COD, NH_4Cl 38.2, KH_2PO_4 43.9, $K_2HPO_4 \cdot 3H_2O$ 73.6, $MgSO_4$ 75, $FeCl_3$ 5.4 and trace element solution (in ug/L): H_3BO_3 2.5, $ZnCl_2$ 2.5, $CuCl_2$ 1.5, $MnCl_2 \cdot 4H_2O$ 5.1, $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ 1.1, EDTA 50, $AlCl_3$ 2.5, $CoCl_2 \cdot 6H_2O$ 1.6, and $NiCl_2$ 2.5. During the 230-day operation of reactor, the sludge concentration was around 6 g/L with a sludge volume index of 20 mL/g.

2.2. Experimental design

After 50 days of operation, EBPR granules with a phosphorus removal efficiency over 92% became matured and stabilized. The granules were yellow in color with a size among 1–2 mm, and white precipitates accumulated in the granule cores (Fig. S1). In order to investigate the effect of Ca^{2+} on the metabolic pathway of PAOs, the influent Ca^{2+} concentration was gradually increased from 28 mg/L (Day 60 to 84) to 42 mg/L (Day 85 to 111), 79 mg/L (Day 112 to 141) and 96 mg/L (Day 142 to 170) to examine the metabolic shift of PAOs, and finally reduced to about 15 mg/L (after Day 171) to examine the recovery of PAO metabolic pathway. The Ca^{2+} concentration in tap water was determined to be about 15 mg/L, and the additional $CaCl_2$ was dosed into the tap water to obtain the preset Ca^{2+} concentration in the influent. During the whole experimental period, the ratio of COD/P, and the influent concentrations of Mg and K were kept constant.

2.3. Characterization of the precipitates inside granules

Phosphorus $L_{2,3}$-edge X-ray absorption near-edge structure (P $L_{2,3}$-edge XANES) spectrum, X-ray diffraction (XRD) and X-ray photoelectron spectroscopy (XPS) were used to qualitatively determine the phosphorus forms of the precipitates in the granular sludge. Before analysis, the granules were crushed and fully grinded, then the mixture was re-suspended in distilled water and rinsed repeatedly to wash out the organic parts, and finally the remaining white mineral powders were freeze-dried. Such a treatment would effectively reduce the interference of organic matters on the analysis, and thus could avoid the form changes of those original minerals comparing with the heating process (Mañas et al., 2011; Lin et al., 2012). P $L_{2,3}$-edge XANES measurements of precipitates and phosphate standards [hydroxyapatite, amorphous calcium phosphate (ACP), brushite and struvite] were performed at soft X-ray magnetic circular dichroism beamline, National Synchrotron Radiation Laboratory (NSRL), Hefei, China. All phosphate standards and samples were finely grounded and then used for the measurement. The monochromatic X-ray light was provided between 100 and 1000 eV, and its flux was over 10^8 photons/s with a resolving power of 1000 at 1000 eV by using a plane varied-line-space grating monochromator. P $L_{2,3}$-edge XANES spectra were recorded between 130 and 160 eV with a step size of 0.2 eV. The data were recorded in the total electron yield mode by collecting sample drain current. No background correction was applied. XRD analysis was performed to examine the crystal shape with a Rigaku TTR-III diffractometer (Rigaku Co., Japan), with data collection from 5° to 70° in 2θ. XPS analysis was carried out on ESCALAB-250 instrument (Thermo Fisher Scientific Inc., USA) with Mg$K\alpha$ radiation to explore the chemical compositions.

2.4. Analysis of microbial populations

Fluorescence in situ hybridization (FISH) analysis was performed to track the population dynamics of PAOs and GAOs in the EBPR reactor. The granules cultivated at various influent Ca^{2+} concentrations were sampled at the end of the aerobic phase and then crushed. After washing with deionized water, sludge samples were fixed with 4% fresh paraformaldehyde solution for 3 h at 4 °C. DAPI (4′,6-diamidino-2-phenylindole) was used for staining all bacteria in this work. Besides, the fluorescently labeled oligonucleotide probes, PAOmix for PAOs (a mixture of probes PAO462,

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