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The long-term effect of carbon source on the competition between polyphosphorus accumulating organisms and glycogen accumulating organism in a continuous plug-flow anaerobic/aerobic (A/O) process

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ABSTRACT

Laboratory experiments were conducted in a continuous plug-flow anaerobic/aerobic (A/O) process to kinetically investigate the long-term effect of the different carbon sources (i.e., acetate, acetate/propionate, propionate and glucose) on the competition between polyphosphorus accumulating organisms (PAOs) and glycogen accumulating organisms (GAOs). It was found that propionate was more benefit than acetate for PAOs even in the A/O process, and PAOs enriched with acetate were readily able to metabolize propionate without the requirement of adaptation. Glucose gave GAOs metabolic advantage in the PAOs–GAOs competition, which thereby worsened the EBPR performance. Nevertheless, the EBPR capacity could recover by returning carbon to acetate, with the acclimation time of approximately 2-SRTs. This suggests that the varying of carbon can be an effective approach to provide PAOs a competitive advantage over GAOs. Additionally, MLVSS/MLSS could indicate the shift of the microorganism between GAOs and PAOs, but it was not as precise as the biomass-P content.

1. Introduction

Enhanced biological phosphorus removal (EBPR) process is one of the most economical and sustainable methods for phosphorus removal from wastewater (Metcalf and Eddy, 2003; Pijuan et al., 2004; Broughton et al., 2008), but at times, its performance is not as reliable as it should be. Deterioration of the EBPR has been observed in laboratory-scale systems as well as wastewater treatment plants (WWTPs) and this has been attributed to the variations of the carbon source in the influent (Tasli et al., 1997; Hollender et al., 2002; Oehmen et al., 2004; Li et al., 2008). Recently, more attention has been paid to the type of carbon source in the wastewater, especially the presence of glucose on EBPR failure, most of which were believed to be attributed to occurrence of glucose accumulating organisms (GAOs) (Mino et al., 1995, 1998; Saunders et al., 2003; Thomas et al., 2003; Oehmen et al., 2007).

In EBPR processes, GAOs can compete for carbon substrate against PAOs. The metabolism of GAOs is proposed to be similar to that of PAOs, except that glycogen serves as the intracellular energy pool for the anaerobic substrate uptake instead of poly-P (Mino et al., 1995, 1998; Hollender et al., 2002). In most previous studies, acetate is usually chosen as the sole carbon source focused on EBPR systems and has been documented to yield robust and stable P removal; whereas, glucose stimulates the proliferation of GAOs and is prone to deteriorate the EBPR performance. Nevertheless, the results of the previous studies are not always consistent, as successful EBPR in the presence of glucose has also been reported in the literature (Carucci et al., 1999; Sudiana et al., 1999; Jeon et al., 2001).

Further, the activated sludge systems generally involve in a much more diverse range of substrates other than acetate, and propionate, butyrate, valerate and other volatile fatty acids (VFAs) may also be present in real wastewaters (Oehmen et al., 2007). Recently, therefore, there has been an increased interest in the impact of propionate, and other substrates on EBPR performance (Thomas et al., 2003; Chen et al., 2004; Oehmen et al., 2006; Li et al., 2008). These studies stated that propionate may be a more favorable substrate than acetate for successful EBPR performance. Whereas, another group of GAOs, Alphaproteobacteria, have been observed to actively take up propionate at substantial rates (Oehmen et al., 2005, 2006; Meyer et al., 2006; Dai et al., 2007) and have the capacity to compete with PAOs for propionate, leading to the deterioration of P removal performance, which therefore, raising the

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questions concerning which substrate actually benefit the steady-operation of the EBPR systems.

So far most of the experimental studies focused on EBPR were conducted in the anaerobic–oxic sequencing batch reactor (A/O-SBR) and the carbon source influence in the continuous-flow A/O process has not been well characterized; particularly, the role of GAOs in the continuous plug-flow system has received little attention. As the different reactor configurations and hydraulic flow characteristics, the response of EBPR for the continuous-flow systems to varying carbon source may be different from the batch system, especially in terms of the competition between PAOs and GAOs.

Our work, therefore, used the continuous-flow A/O EBPR process to kinetically investigate the effect of carbon source, i.e., acetate, the acetate/propionate mixture, propionate and glucose, on the phosphorus removal as well as the competition mechanism between PAOs and GAOs. Further, since there are little experimental data available to show whether A/O process can replenish its phosphorus removal capacity once GAOs dominate the system, the control strategy like varying carbon source to provide PAOs with a selective advantage over GAOs was also examined. Batch tests were conducted to evaluate the carbon source effect on the anaerobic and aerobic metabolisms regarding stoichiometry and kinetics.

2. Methods

2.1. Process setup and operation

A laboratory-scale plug-flow anaerobic–aerobic (A/O) reactor was made from plexiglass with a working volume of 32 L. There were eight compartments for the A/O process, separated by baffles to achieve the anaerobic and aerobic zones. The first four compartments were operated as the anaerobic phases, and the following four ones operated as the aerobic phases. The volume of each compartment was same with a dimension of 120 mm × 80 mm × 420 mm (H × W × D). A mechanical stirrer provided mixing in each anaerobic compartment to keep the biomass in suspension and an air pump supplied air through a porous stone diffuser which was installed at the bottom of each aerobic compartment. Additionally, an air flow meter was used for controlling the airflow rate and dissolved oxygen (DO) concentrations in the reactor. The biomass of the A/O system settled in a cylindrical clarifier with a total volume of 15 L. The influent flow (around 6 L/h) and the sludge recycled flow (120–130% of influent flow rate) were controlled by two peristaltic pumps. Mixed liquor suspended solid (MLSS) was controlled in the range of 3500–4200 mg/L, resulting in the solid retention time (SRT) of 6–8 days.

The EBPR biomass was enriched using acetate as the sole carbon source. After the steady P removal achieved, the A/O process operated with different carbon source thereafter, equivalently on a COD basis. On day 62–91, acetate was continued to be the sole carbon source; from day 92 to 121, the A/O process was fed with the mixture of acetate and propionate (C-mol = 1:2); on day 122–157, propionate was used as the sole carbon source; from the following days of 158–189, glucose was used as the sole carbon source; finally, during days 190–220, the fed carbon source was returned to acetate in order to recover the net P-removal capacity.

2.2. Synthetic wastewater and activated sludge

Synthetic wastewater used in this study contained (per liter): COD (carbon sources: acetate, the mixed acetate and propionate (1:2), propionate and glucose) 350 mg; KH_2PO_4: 15 mg; NH_4Cl: 20 mg; $MgSO_4{\cdot}7H_2O$: 10 mg; $CaCl_2$: 14 mg. Additionally, 0.3 mL of mineral salt solution was added per liter of synthesis wastewater. Each liter of mineral salt solution was composed of 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 ethylene-diaminetetraacetic acid (EDTA). Temperature was maintained at 18–25 °C. The A/O process was inoculated with the activated sludge from Quyang Sewage Treatment Plant (Shanghai, China), where an oxidation process is implemented.

2.3. Batch tests

Supplementary batch tests were carried out in 500 mL jacketed magnetically stirred glass reaction vessels. The sludge (500 mL) for the batch tests was taken from the final aerobic compartment of the A/O system on day 76, 116, 145, 180 and 217, respectively, and was washed three times with tap water before transferred into the reaction vessels. Then, 400 mL of synthetic feed was added as a pulse at the beginning of the cycle with the initial COD and PO_4^{3-}—P concentrations of approximately 350 mg/L and 13.0 mg/L, respectively. The mixed liquor was incubated for 2 h under anaerobic condition by injecting nitrogen gas above the water surface and then was exposed to aerobic conditions for 3 h. The MLSS was maintained at around 3700–3800 mg/L and the pH was kept $\leqslant$8.0 by adding $NaHCO_3$ solution.

The liquid and solid-phase samples were taken for chemical analysis with sampling intervals of 5–10 min during the first 30 min of the anaerobic period and aerobic period and each 30 min afterwards. Three cycle studies were carried out for the individual sludge acclimated with the different carbon sources.

2.4. Analytical methods

The liquid samples were immediately filtered through Millipore filter units (0.45 μm pore size) for the analysis of phosphate (PO_4^{3-}—P). PO_4^{3-}—P, NH_4^+—N, NO_3^-—N, NO_2^-—N, MLSS, mixed liquor volatile suspended solid (MLVSS) and biomass-P content were analyzed in accordance with standard methods (APHA, 1998). For the COD test, Merck COD reagents were used (Merck; Germany). Glycogen was determined by the method of Jenkins et al. (2003). On-line monitoring was installed in the A/O reactor with DO (WTW inoLab Oxi level 2 oxygen meters), pH and ORP (WTW pH/Oxi340i) sensors. The temperature was measured by a probe incorporated in the DO probe.

3. Results

After 61 days of operation, the steady P release and uptake were achieved in the A/O process. Then, the experiments were performed under the same operational conditions but with the four different substrates, i.e., acetate, the acetate/propionate mixture, propionate and glucose. Acetate was returned to be the substrate after the addition of glucose.

3.1. Phosphorus removals in the A/O process with the different carbon source

The P removal efficiencies of the plug-flow A/O process depended greatly on the carbon sources (Fig. 1). Net P removals were observed in all experimental periods (Fig. 1a). The highest P removal of 95 ± 1.24% was achieved when propionate as the sole carbons source and the poorest P removal of 46 ± 2.63% was observed when using glucose as the sole carbon source (Fig. 1b). The effluent PO_4^{3-}—P concentrations at steady state was 2.1 ± 0.74, 1.3 ± 0.33, 0.7 ± 0.18, 7.1 ± 0.46 and 1.5 ± 0.34 mg/L, respectively, with the