Competition between polyphosphate- and glycogen-accumulating organisms in biological phosphorus removal systems – effect of temperature

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Abstract
This study demonstrated that temperature is an important factor in determining the outcome of competition between polyphosphate-accumulating organisms (PAOs) and glycogen-accumulating non-poly-P organisms (GAOs) and the resultant stability of enhanced biological phosphorus removal (EBPR) systems. At 20°C and a 10-day sludge age, PAOs were dominant in the anaerobic/aerobic (A/O) SBR, however, at 30°C and a 10-day sludge age, GAOs were dominant in the A/O SBR. For kinetic batch studies, the anaerobic specific acetate uptake rate of GAO-dominated sludge ($1.34 \times 10^{-3}$ mg C/mg VSS·minute) was higher than the rate of PAO-dominated sludge ($0.89 \times 10^{-3}$ mg C/mg VSS·minute) at 30°C, leading to the eventual failure of EBPR processes at high temperatures.

Keywords
Enhanced biological phosphorus removal (EBPR); glycogen-accumulating non-poly-P organisms (GAOs); polyphosphate-accumulating organisms (PAOs); temperature

Introduction
The enhanced biological phosphorus removal (EBPR) system treating municipal wastewater is generally stable with regard to phosphorus removal. However, failure of phosphorus removal has been reported due to unknown reasons (Cech and Hartman, 1990, 1993; Satoh et al., 1994). A particular type of microorganism, referred to as glycogen-accumulating non-poly-P organisms (GAOs) (Mino et al. 1998), can take up organic substrate anaerobically without phosphorus release and is believed to relate to the deterioration of EBPR systems. Maszenan et al. (1998) isolated three gram negative tetrad cocci from different wastewater treatment plants and found those isolates have similar characteristics to the original isolate of the “G-bacteria” (now referred to as GAOs) described by Cech and Hartman (1990). Definite conditions for the proliferation of GAOs have not been fully determined and the mechanism of competition between PAOs and GAOs is not fully understood. Liu et al. (1997) suggested the approach to increase the GAO population by limiting phosphorus in the feed to the minimum requirement for biomass synthesis. Bond (1997) and Filipe (1999) reported that the pH in the anaerobic stage plays an important role in determining the dominant species between PAOs and GAOs. In this study, the effect of temperature (20 and 30°C) on competition between PAOs and GAOs was investigated by using laboratory-scale sequencing batch reactors (SBRs).

Materials and methods

SBR operation
Two laboratory-scale SBRs were operated as anaerobic-aerobic (A/O) activated sludge systems at different temperatures (20 and 30°C). The SBRs were operated in a 6 hour cycle – fill (5 minutes) during anaerobic phase (90 minutes), aerobic phase (220 minutes), settling (30 minutes), decant (15 minutes), and idle (5 minutes). The mixed liquor volume in the SBRs at the end of the fill of synthetic wastewater (3 L) was 6 L, and 3 L of treated supernatant was withdrawn after settling. Daily 0.6 L of mixed liquor was withdrawn at
the end of the aerobic phase to maintain 10 days of sludge age for both SBRs. The pH in the SBRs was controlled at 7.0 ± 0.5 throughout the experiments. The ingredients of synthetic wastewater included: CH₃COONa (380 mg/L), NH₄Cl (80 mg/L), KH₂PO₄ (42–128 mg/L), MgCl₂·6H₂O (312 mg/L), CaCl₂·2H₂O (78 mg/L), FeCl₂·6H₂O (1.26 mg/L), ZnSO₄·7H₂O (3.5 mg/L), yeast extract (6 mg/L), and peptone (35 mg/L). The total measured COD in the synthetic wastewater was 330 mg/L including 300 mg/L of readily biodegradable COD (CODrbi) contributed from sodium acetate. To avoid nitrification in the SBRs, 1 mg/L of allylthiourea was added to the synthetic wastewater. The activated sludge seeds for the SBRs were obtained from Madison Metropolitan Sewerage District (MMSD) WWTP (Madison, Wisconsin), which is currently removing phosphorus biologically by using a modified UCT process.

**Analytical methods**

Acetate was purified through treatment with calcium hydroxide and cupric acid and then analyzed by HPLC. Analytical methods of polyhydroxyalkanoates (PHAs) [including 3-hydroxybutyrate (HB) and 3-hydroxyvalerate (HV)] can be found elsewhere (Liu et al., 1996). The cellular glycogen was extracted and determined by the following phenol reaction (Gerhardt et al., 1994).

**Results and discussion**

At 20°C and a 10-day sludge age (Experiment I), PAOs were dominant in the A/O SBR and able to remove phosphorus as high as 40 mg-P/L in the influent (CODrbi/P = 300/40). The biomass phosphorus content of the PAO-dominated sludge was 18% of mixed liquor suspended solids (MLSS). Variations of influent and effluent phosphorus concentrations, biomass phosphorus content, and anaerobic phosphorus release in Experiment I are shown in Figure 1.

At 30°C and a 10-day sludge age (Experiment II), phosphorus was not detected during the first 15 days; however, EBPR in the SBR ceased at day 17. After day 19, the influent phosphorus concentration was decreased to 10 mg-P/L (CODrbi/P = 300/10) and the average effluent phosphorus concentration was 8.8 mg-P/L between days 20 to 120. The biomass phosphorus content decreased from 10.4% (based on MLSS) at day 16 to less than 1% (based on MLSS) at day 26. No significant anaerobic phosphorus release was observed after day 26. The low biomass phosphorus content and no significant phosphorus release/uptake phenomena observed in the SBR after day 26 indicated that EBPR had ceased. The variations of influent and effluent phosphorus concentrations, and biomass phosphorus content in Experiment II are shown in Figure 2. In spite of the deterioration of EBPR in the SBR, complete acetate uptake under anaerobic conditions was observed. In addition, anaerobic accumulation of PHAs and consumption of glycogen were also observed in the biomass samples. Figure 3 presents the SBR profile of acetate and phosphorus concentration as well as biomass storage polymers in a 6 hour cycle. Figure 4 shows the characteristic morphology of the dominant microorganisms in the SBR. These microorganisms, arranged in distinctive tetrads, are very similar in morphology to the GAOs reported by Liu et al. (1996) and Maszenan et al. (1998). Therefore, GAOs are believed to be dominant in the A/O SBR after 20 days of competition with PAOs at 30°C and a 10-day sludge age.

In addition to the long-term experiments, batch experiments were conducted to investigate the anaerobic acetate uptake kinetics of PAO- and GAO-dominated sludge (taken from Experiment I and II, respectively) at different temperatures (10, 20, and 30°C). The results are shown in Figures 5 and 6 and summarized in Table 1. The results indicate that the anaerobic specific acetate uptake rate of GAO-dominated sludge (1.34 × 10⁻³ mg C/mg
VSS·minute) is higher than the rate of PAO-dominated sludge (0.89 × 10⁻³ mg C/mg VSS·minute) at 30°C, leading to the eventual failure of EBPR processes at high temperatures.
Conclusions
This study has demonstrated that temperature is an important factor in determining the outcome of competition between PAOs and GAOs and the resultant stability of EBPR. GAOs were able to out-compete PAOs at high temperature (30°C) and dominated in the EBPR system, possibly due to a kinetic advantage in anaerobic acetate uptake.

References