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The effect of free nitrous acid on key anaerobic processes in enhanced biological phosphorus removal systems

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HIGHLIGHTS

- FNA has a detrimental effect on the anaerobic metabolism of both PAOs and GAOs.
- FNA has a stronger adverse effect on acetate uptake by PAOs than by GAOs.
- FNA causes faster depletion of the anaerobic energy pool of PAO than that of GAOs.
- FNA may potentially provide a competitive advantage to GAOs over PAOs.

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ABSTRACT

In this study, the effect of nitrite/FNA on the anaerobic metabolism of polyphosphate accumulating organisms (PAOs) and glycogen accumulating organisms (GAOs) is investigated. The results clearly show that FNA has a detrimental effect on the acetate uptake rate by both PAOs and GAOs, but this adverse effect is much stronger on PAOs than on GAOs. Also, when FNA was increased, phosphate release to acetate uptake ratio by PAOs increased substantially (250–300% compared to control), which was accompanied by decreases (40–60%) in glycogen degradation and PHA production to VFA uptake. In contrast, these ratios for GAOs remained constant or increased slightly towards the highest FNA concentration applied. These results indicate that the anaerobic metabolism of PAOs is more adversely affected than that of GAOs when FNA is present. This might provide a competitive advantage to GAOs over PAOs in enhanced biological phosphorus removal systems when nitrite is present.

1. Introduction

Removal of phosphorus from wastewater is necessary for avoiding eutrophication. Enhanced biological phosphorus removal (EBPR) is widely accepted as one of the most economical and sustainable processes to remove phosphorus from wastewater for its low operation costs and low sludge production compared to chemical phosphorus removal. The micoorganisms responsible for EBPR processes are known to be polyphosphate accumulating organisms (PAOs). They can take up carbon sources, primarily volatile fatty acids (VFAs), in the anaerobic phase and store them in the form of poly-hydroxy-alkanoates (PHAs). PAOs gain energy primarily from the degradation of their intracellular poly-phosphate (Poly-P), which is released to the bulk liquid as orthophosphate. In the subsequent anoxic or aerobic phase, PAOs grow and take up orthophosphate to recover their Poly-P levels by using the stored PHA as the carbon and energy sources. Phosphorus removal is achieved by withdrawing excess sludge at the end of the aerobic phase, when PAO cells contain high levels of Poly-P. As a competitor to PAOs, glycogen accumulating organisms (GAOs) can also be developed under alternating anaerobic and aerobic conditions in EBPR systems. However, the GAO metabolism does not involve anaerobic P release and subsequent aerobic (or anoxic) P-uptake. Anaerobically, the energy for GAOs is primarily generated by the degradation of their intracellular glycogen pools. Under the subsequent aerobic or anoxic conditions, GAOs oxidize PHA for cell growth and glycogen replenishment without P-uptake (Liu et al., 1994). Therefore, GAOs are able to compete with PAOs for the same carbon source, which may lead to EBPR deterioration. However, the PAO–GAO competition in an EBPR system is not fully understood at present, and indeed many factors may play a role (Oehmen et al., 2007).

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Biological phosphorus removal typically occurs concomitantly to nitrogen removal in most modern biological wastewater treatment plants (WWTPs). Nitrite is an intermediate in both nitrification ($NH_4^+ \rightarrow NO_2^- \rightarrow NO_3^-$) and denitrification ($NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$), but in general does not accumulate to significant levels in conventional WWTPs. However, nitrogen removal via the nitrite pathway ($NH_4^+ \rightarrow NO_2^- \rightarrow N_2$) is increasingly used driven by energy and carbon savings (see Zhou et al., 2011). In such systems, nitrite often accumulates. Nitrite accumulation at the end of the aerobic zone/phase to 12.3–22.6 mg N/L has been reported in reactors treating domestic wastewater (Yang et al., 2007; Zeng et al., 2011) and accumulation to even higher levels (up to 40 mg N/L) has been observed during abattoir wastewater treatment (Pijuan et al., 2010a).

Nitrite accumulation has been reported to negatively affect aerobic/anoxic P-uptake (Hu et al., 2003; Meinhold et al., 1999; Saito et al., 2004; Yoshida et al., 2006). Free nitrous acid (FNA), rather than nitrite, has recently been suggested to be the true inhibitor on many microorganisms involved in biological wastewater treatment (Zhou et al., 2011), including the aerobic and anoxic P-uptake by PAOs (Pijuan et al., 2010b; Zhou et al., 2007, 2008). Ye et al. (2010) reported that FNA also inhibited the aerobic metabolism of GAOs although this inhibition was milder than that on PAOs. It was therefore postulated that FNA may disadvantage PAOs in their competition with GAOs in EBPR systems (Pijuan et al., 2010b). To fully assess the FNA implications on the PAO–GAO competition, the effect of nitrite/FNA on the anaerobic metabolism of both groups needs to be investigated. This is because the accumulated nitrite can be recirculated or leaked into the anaerobic phase/cycle (Pijuan et al., 2010a; Zeng et al., 2011). Any impact of nitrite/FNA on the relative capability of PAOs and GAOs to take up carbon sources under anaerobic conditions would have a profound effect on their competition in an EBPR system, directly impacting on the performance and stability of the EBPR process.

Nitrite/FNA may potentially have two distinct effects on the anaerobic metabolism of PAOs and GAOs. First of all, FNA may have direct inhibitory effects on the normal anaerobic reactions performed by these organisms, analogous to its inhibitory effects on other metabolic processes. Secondly, both PAOs and GAOs are known to be able to denitrify (Kuba et al., 1993; Zeng et al., 2003; Coma et al., 2012). Therefore, nitrite will likely be used as a terminal electron acceptor by these organisms. The latter process will consume VFAs, thus indirectly impacting on the anaerobic processes, as has been previously observed when nitrate was leaked to the anaerobic zone/period (Kuba et al., 1993; Zeng et al., 2011). To fully assess the impact of FNA on the anaerobic processes of PAOs and GAOs, both of the above two types of effects should be studied.

In this study, the direct inhibitory effects of nitrite/FNA on the anaerobic metabolism of PAOs and GAOs are investigated. To distinguish these effects from those caused by substrate competition, highly enriched cultures of *Candidatus* "Accumulibacer phosphatis" (a well known PAO, henceforth referred to as *Accumulibacer*) and *Candidatus* "Competibacter phosphatis" (a well known GAO, henceforth referred to as *Competibacter*) that were unadapted to nitrite were used. We hypothesized that such cultures would not be able to reduce nitrite during a short-term (2 h) exposure to nitrite, and therefore the true inhibitory effects of FNA on the normal anaerobic reactions by PAOs and GAOs would be revealed. A series of batch tests were carried out using these cultures at pre-designed pH levels and nitrite concentrations. The VFA uptake rate, PHA production, glycogen consumption, and phosphate release (for PAOs only) were measured and analyzed against the FNA concentrations applied.

2. Methods

2.1. Sludge source and reactor operation

A sequencing batch reactor (SBR) fed with synthetic wastewater containing volatile fatty acids and orthophosphate was used to enrich PAOs. The reactor had a working volume of 4 L and was operated with a cycle time of 6 h consisting of 130 min anaerobic, 160 min aerobic, 65 min settling and decanting, and 5 min anaerobic idle periods. Allylthiourea (ATU) was added in the feed (7.94 mg/L in the reactor after feeding) to inhibit nitrifiers. In each cycle, 1 L of synthetic wastewater (containing 40 mg P/L phosphate, and 600 mg COD/L of acetate or propionate) was fed to the reactor in the first 6 min of the anaerobic period, resulting in a hydraulic retention (HRT) time of 24 h. The sole carbon source in the synthetic feed was alternated between acetate and propionate with a switching frequency of two sludge ages (16 days), in order to provide further selective advantages to PAOs over GAOs (Lu et al., 2006). Further explanation of the feeding strategy and more details of the reactor operation can be found in Lu et al. (2006). The SBR was displaying excellent EBPR performance (>99% P removal) when the batch experiments described below were carried out.

Another SBR fed with synthetic wastewater was used to enrich GAOs. Acetate was used as the sole carbon source. The reactor, with a working volume of 8 L, was operated with a cycle time of 6 h consisting of 120 min anaerobic, 180 min aerobic, 2.5 min wasting and 57.5 min settling and decanting periods. In each cycle, 2 L of synthetic wastewater containing 200 mg COD/L of acetate was fed to the reactor in the first 5 min of the anaerobic period, resulting in an HRT of 24 h. ATU was also supplied in the feed (0.53 mg/L in the reactor after feeding) to inhibit nitrifiers. The sludge age was 8 days. More details of the feed and operational conditions can be found in Ye et al. (2010).

2.2. Batch experiments

Batch tests were carried out with both sludges to examine the impact of nitrite/FNA on anaerobic carbon (C) uptake, phosphate (P) release, PHA production and glycogen degradation. Five sets of batch tests were conducted with each sludge. Each set included a blank test, where no nitrite was added, and three other tests with different nitrite concentrations. The pH was the same in all the batch experiments performed within the set. In each set, 1 L of mixed liquor was withdrawn from the corresponding SBR at the end of an aerobic period and evenly distributed between four batch-reactors (reactor volume 300 mL) operated in parallel. A mixed liquor sample of 50 mL was also taken from the SBR at the same time for the analysis of glycogen and PHA in triplicates. Different volumes of a nitrite stock solution were added to the batch reactors at the beginning of the experiment, which resulted in initial concentrations of nitrite varying between 0 and 93.8 mg N/L, as summarized in Tables 1 and 2. Each anaerobic test lasted for 2 h, during which pH was kept approximately constant (±0.1) at a pre-designed set-point (see Tables 1 and 2), through addition of 0.5 M HCl or 0.2 M NaOH. Sodium acetate solution was added at the beginning of each test, resulting in an initial COD concentration of approximately 200 mg/L. Nitrogen was sparged during the entire test to ensure anaerobic conditions.

The concentration of FNA, the key factor tested in this study, ranged between 0 and 22.2×10^{-3} mg HNO_2–N/L (Tables 1 and 2), which was achieved by varying the nitrite concentration and pH. The FNA concentration was calculated using the formula $S_{N-NO_2^-}/K_a \times 10^{pH}$ with the K_a value determined using the formula $K_a = e^{-2,300/(273+T)}$ for a given temperature T (°C) (Anthonisen