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Survival strategies of polyphosphate accumulating organisms and glycogen accumulating organisms under conditions of low organic loading

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HIGHLIGHTS

- Extended periods of low organic loading impact the PAO–GAO competition.
- PAOs possess higher anaerobic activity decay.
- GAOs possess higher biomass decay and aerobic activity decay.
- PAOs survive better under low organic loading conditions.
- PAOs tended to maintain a higher residual PHA reserve than GAOs.

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ABSTRACT

Enhanced biological phosphorus removal (EBPR) is usually limited by organic carbon availability in wastewater treatment plants (WWTPs). Polyphosphate accumulating organisms (PAOs) and glycogen accumulating organisms (GAOs) were operated under extended periods with low organic carbon loading in order to examine its impact on their activity and survival. The decrease in organic carbon load affected PAOs and GAOs in different ways, where the biomass decay rate of GAOs was approximately 4 times higher than PAOs. PAOs tended to conserve a relatively high residual concentration of polyhydroxyalkanoates (PHAs) under aerobic conditions, while GAOs tended to deplete their available PHA more rapidly. This slower oxidation rate of PHA by PAOs at residual concentration levels enabled them to maintain an energy source for aerobic maintenance processes for longer than GAOs. This may provide PAOs with an advantage over GAOs in surviving the low organic loading conditions commonly found in full-scale wastewater treatment plants.

1. Introduction

In enhanced biological phosphorus removal (EBPR) wastewater treatment processes, polyphosphate accumulating organisms (PAOs) and glycogen accumulating organisms (GAOs) compete for the limited supply of organic carbon sources such as volatile fatty acids (VFAs) (Oehmen et al., 2007). Mixed culture enrichments containing PAOs and/or GAOs have been often used to study operational and environmental factors that impact the competition between these two groups of organisms, such as carbon source composition, pH, temperature, sludge retention time, influent acetate to phosphorus (P) ratio and substrate feeding rate (Lopez-Vazquez et al., 2009; Tu and Schuler, 2013; Whang and Park, 2006). Nevertheless, the VFA load used in lab-scale systems is substantially higher than the typical load received at most full-scale wastewater treatment systems. The reason for this is that it is often desirable to achieve highly enriched cultures at the expense of the elimination of other organisms, such as autotrophs and ordinary heterotrophs, thus the VFA load under anaerobic conditions is increased to maintain high selectivity of the culture for PAOs and/or GAOs.

The efficiency of the EBPR process is usually limited by the content of VFAs present in domestic wastewater (Barnard, 1984). Although the VFA content is dependent on the wastewater characteristics, their concentration in domestic wastewater typically lies between 10 and 74 mg COD/L (Siedlecka et al., 2008; Thomas et al., 2003; Zeng et al., 2006). Some studies have focussed on the effect

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of short-term (i.e., 1–3 d) decreases in organic load in an effort to simulate the decrease in organic load that occurs during weekends in many wastewater treatment plants (Ahn et al., 2006; Carucci et al., 1999; Miyake and Morgenroth, 2005; Temmink et al., 1996). Results have shown that after a rapid decrease in the initial organic carbon concentration (from 200–400 mg COD/L to levels between 10 and 100 mg COD/L), the EBPR performance deteriorated, most notably through a decrease in aerobic P uptake. Moreover, Temmink et al. (1996) observed a depletion in the polyhydroxyalkanoates (PHAs) pool, which affected the P uptake, since the aerobic P uptake rate is highly dependent on the level of PHA. Ahn et al. (2006) showed that an abrupt decrease in the organic concentration from 150 to 50 mg/L, PAOs could not adapt themselves to these sudden changes and the EBPR system became unstable. However, these studies did not evaluate the impact of prolonged low carbon loading periods on the metabolism of PAOs and GAOs, and consequently, the effect of this factor on the competition between both organisms. Knowledge about microbial competition under stress conditions (such as low organic loading) is very important for EBPR systems, since it will contribute towards the development of strategies that will promote the proliferation of PAOs and, consequently, improve EBPR performance.

Tu and Schuler (2013) studied the effect of lowering the acetate concentration in an EBPR system through decreasing the feeding rate, while maintaining the total acetate load to the bioreactor (i.e., 200 mg COD/L per cycle). With this approach, it was found that PAOs were able to outcompete GAOs. Nevertheless, the impact of long periods of low total organic carbon loading, such as those normally present in continuous full-scale WWTPs (e.g., with influent in the 10–74 mg COD/L range), has not been previously assessed. Under conditions of low total organic loading, the maintenance processes of PAOs and GAOs become increasingly crucial, since each group of organisms will experience an increased quantity of time in the absence of external carbon sources. This will lead to the consumption of their internal carbon and energy sources: polyhydroxyalkanoates (PHAs) and glycogen, as well as polyphosphate in the case of PAOs.

Previous studies have investigated the effect of long-term starvation on PAOs (21 days) and GAOs (26 days) (Vargas et al., 2013), and have found that PAOs display a faster decay rate as compared to GAOs, while the specific acetate uptake capacity of PAOs is also decreased at a higher rate as compared to GAOs. While these tests focused on the response of each organism to extended periods without external carbon source, it is unclear if a similar response may be expectable under a situation of low organic loading, at a level close to that typically found in full-scale wastewater treatment plants (WWTPs).

In the present study, the effect of prolonged low carbon loading on PAO and GAO metabolism was investigated for the first time in order to understand the impact of this factor on their activity and survival.

2. Methods

In this study, two sequencing batch reactors (SBRs) were operated initially under conditions of high organic concentration (VFA_{in} = 200 mg COD/L) in order to enrich for PAOs in one SBR, and GAOs in the other. Then, each system was subjected to a decrease in the initial VFA concentration, to about 33 mg COD/L. This concentration was chosen since it is within the typical range observed in full-scale WWTPs.

2.1. PAO and GAO parent reactors

Two sequencing batch reactors (SBR) with 2L of working volume were operated to select for PAO and GAO, respectively, and were seeded from the lab-scale PAO and GAO reactors described in Carvalheira et al. (in press). In both SBRs, the cycle consisted of 6 h, with a 2 h anaerobic period, 3 h aerobic period and 1 h settle/decant period. Both SBRs were fed during the first 5 min of the anaerobic phase, obtaining an initial concentration of 200 mg COD/L of carbon source and a phosphate concentration of 20 mg P/L for the PAO reactor and 1 mg P/L for the GAO reactor. In the PAO reactor, a mixture of acetate (HAc) and propionate (HPr) (75–25% HAc–HPr) was used and in the GAO reactor only HAc was fed. This was performed according to previous studies showing that mixed VFA feeds favour the growth of PAO over GAO, as compared to single substrates (Carvalheira et al., 2014; Lopez-Vazquez et al., 2009). The feed solution was composed of 250 mL of solution A and 750 mL of solution B. Solution A contained per litre: 0.59 g NH_4Cl, 0.95 g $MgSO_4{\cdot}7H_2O$, 0.44 g $CaCl_2{\cdot}2H_2O$, 11.7 mg allyl-N thiourea (ATU), 31.7 mg ethylene-diaminetetraacetic (EDTA) and 3.17 mL of a micronutrients solution in both SBRs. Additionally, the PAO SBR also contained 2.55 g $C_2H_3O_2Na{\cdot}3H_2O$ and 270 μL $C_3H_6O_2$ in solution A, while the GAO SBR contained 3.40 g $C_2H_3O_2Na{\cdot}3H_2O$. The micronutrient solution (based on Smolders et al. (1994)) contained per litre: 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 $Na_2MoO{\cdot}2H_2O$, 0.12 g $ZnSO_4{\cdot}7H_2O$, 0.15 g $CoCl_2{\cdot}6H_2O$. Solution B contained per litre: 124.1 mg K_2HPO_4 and 96.8 mg KH_2PO_4 in the PAO reactor and 7.50 mg K_2HPO_4 and 5.86 mg KH_2PO_4 in the GAO reactor.

In both SBRs, the hydraulic retention time (HRT) was 12 h and the solid retention time (SRT) was 8 days. To maintain anaerobic conditions, argon was bubbled into the reactors at a flow rate of approximately 15 mL/min. During the aerobic phase, the dissolved oxygen (DO) concentration was controlled at 2.2 ± 0.4 mg O_2/L for the PAO reactor and 1.9 ± 0.3 mg O_2/L for the GAO reactor, using an on–off valve. The temperature was controlled at 20 ± 1 °C and pH was controlled at 7.3 ± 0.1 for the PAO reactor and 7.0 ± 0.0 for the GAO reactor, by automatic addition of 0.1 M HCl. These conditions were maintained for >3 SRT in each system, 36 and 27 days for PAOs and GAOs, respectively.

Monitoring of the SBRs involved samples taken at various time points for volatile fatty acids (VFAs), phosphorus (P), polyhydroxyalkanoates (PHAs) and glycogen. The total suspended solids (TSS) and volatile suspended solids (VSS) were sampled at the end of the aerobic phase, while fluorescence *in situ* hybridisation (FISH) samples were taken at the end of the anaerobic and aerobic phases.

2.2. PAO and GAO reactors with low organic load

The carbon source concentration was decreased to 32.7 ± 0.9 mg COD/L to study the effect of low organic load on PAO and GAO cultures, maintaining an identical HAc:HPr ratio in each system as indicated in Section 2.1. Due to the low organic load, the SRT was increased to 50 days to prevent biomass washout. Except for the VFA concentrations, the composition of solutions A and B as well as all other operational conditions were maintained identical to the parent reactors. Each system was operated for 29 days under these conditions. Samples taken for monitoring both SBRs were identical to that outlined in Section 2.1.

2.3. Chemical and microbial analysis

The concentration of VFAs in the supernatant was determined by liquid chromatography (HPLC) with an Aminex HPX-87H column (BioRad) and a UV detector. H_2SO_4 (0.005 M) was used as eluent at a flow rate of 0.6 mL/min and the operating temperature was 50 °C. Phosphate was determined by colorimetry, as implemented in a flow segmented analyser (Skalar 5100, Skalar Analytical, The Netherlands). For total phosphate, an acid digestion of a sample