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The effect of pH on the competition between polyphosphate-accumulating organisms and glycogen-accumulating organisms

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Abstract

In enhanced biological phosphorus removal (EBPR) processes, glycogen-accumulating organisms (GAOs) may compete with polyphosphate-accumulating organisms (PAOs) for the often-limited carbon substrates, potentially resulting in disturbances to phosphorus removal. A detailed investigation of the effect of pH on the competition between PAOs and GAOs is reported in this study. The results show that a high external pH (~8) provided PAOs with an advantage over GAOs in EBPR systems. The phosphorus removal performance improved due to a population shift favouring PAOs over GAOs, which was shown through both chemical and microbiological methods. Two lab-scale reactors fed with propionate as the carbon source were subjected to an increase in pH from 7 to 8. The phosphorus removal and PAO population (as measured by quantitative fluorescence in situ hybridisation analysis of "*Candidatus* Accumulibacter phosphatis") increased in each system, where the PAOs appeared to out-compete a group of *Alphaproteobacteria* GAOs. A considerable improvement in the P removal was also observed in an acetate fed reactor, where the GAO population (primarily "*Candidatus* Competibacter phosphatis") decreased substantially after a similar increase in the pH. The results from this study suggest that pH could be used as a control parameter to reduce the undesirable proliferation of GAOs and improve phosphorus removal in EBPR systems.

Keywords: Enhanced biological phosphorus removal (EBPR); Polyphosphate-accumulating organisms (PAO); Glycogen-accumulating organisms (GAO); Volatile fatty acids (VFA); pH; Fluorescence in situ hybridisation (FISH)

1. Introduction

Enhanced biological phosphorus removal (EBPR) is an activated sludge process operated with sequential anaerobic and aerobic periods. A group of bacteria known as polyphosphate-accumulating organisms (PAOs) are able to take up volatile fatty acids (VFAs) anaerobically and convert them to intracellular poly-β-hydroxyalkanoates (PHAs). PAOs gain the energy and reducing power required for anaerobic VFA uptake and conversion to PHA through the hydrolysis of their intracellularly stored polyphosphate (poly-P) and glycogen (Pereira et al., 1996; Mino et al., 1998). Aerobically, PAOs oxidise PHA to gain energy for growth, glycogen

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Nomenclature

PAO	polyphosphate-accumulating organism
GAO	glycogen-accumulating organism
VFA	volatile fatty acid
PHA	poly-β-hydroxyalkanoate
PHB	poly-β-hydroxybutyrate
PHV	poly-β-hydroxyvalerate
PH2MV	poly-β-hydroxy-2-methylvalerate
SBR	sequencing batch reactor
FISH	fluorescence in situ hybridisation
P/VFA	anaerobic phosphorus release to VFA uptake ratio
Glycogen/VFA	anaerobic glycogen hydrolysis to VFA uptake ratio
PHB/VFA	anaerobic PHB production to VFA uptake ratio
PHV/VFA	anaerobic PHV production to VFA uptake ratio
PH2MV/VFA	anaerobic PH2MV production to VFA uptake ratio
PHA/VFA	total anaerobic PHA production to VFA uptake ratio

replenishment and P uptake. "*Candidatus* Accumulibacter phosphatis" (henceforth referred to as *Accumulibacter*), has been shown to display PAO activity as described above (Hesselmann et al., 1999; Crocetti et al., 2000). Using fluorescence in situ hybridisation (FISH) techniques, *Accumulibacter* has frequently been found to dominate many lab-scale EBPR cultures (Liu et al., 2001; Onuki et al., 2002; Pijuan et al., 2004; Oehmen et al., 2005b) and has also been observed in abundance in full-scale wastewater systems (Saunders et al., 2003).

Glycogen-accumulating organisms (GAOs) are a group of bacteria capable of competing with PAOs for anaerobic VFA uptake in EBPR systems. Like PAOs, GAOs take up VFAs anaerobically and convert them to PHAs, however, they hydrolyse glycogen as their sole source of energy for this process (Mino et al., 1994; Satoh et al., 1994). GAOs do not perform anaerobic P release and aerobic P uptake transformations, hence they do not contribute to P removal. "*Candidatus* Competibacter phosphatis" (henceforth referred to as *Competibacter*) (Crocetti et al., 2002) has been shown to display the GAO phenotype. *Competibacter* has been frequently enriched in lab-scale reactors fed with acetate as the sole carbon source (Liu et al., 2001; Crocetti et al., 2002; Zeng et al., 2003; Oehmen et al., 2005b), and its presence has also been shown in full-scale wastewater treatment plants (Crocetti et al., 2002; Kong et al., 2002; Saunders et al., 2003). The GAO phenotype has recently been displayed in anaerobic–aerobic activated sludge systems without the detection of *Competibacter*. This novel group of GAOs were shown to be members of the *Alphaproteobacteria* phylum (Beer et al., 2004; Wong et al., 2004; Oehmen 2005), and were observed to exhibit a tetrad-type morphology.

Since GAOs are capable of anaerobic VFA uptake, as are PAOs, it is desirable to minimise or eliminate the growth of GAOs in EBPR systems. Previous studies have suggested that pH may be an important factor that affects the competition between PAOs and GAOs. In some cases, a higher level of phosphorus removal has been observed after a pH increase (Filipe et al., 2001b; Jeon et al., 2001; Schuler and Jenkins 2002; Serafim et al., 2002), and it has been hypothesised that a higher pH is more beneficial for PAOs and less favourable for GAOs.

In this study, the effect of pH on EBPR performance and the competition between PAOs and GAOs are investigated. Two distinct groups of GAOs, namely the *Competibacter* GAOs and the alphaproteobacterial GAOs, were enriched using acetate and propionate, respectively, as the carbon sources. The ability of PAOs to compete with these different groups of GAOs at neutral (~7) and elevated (~8) pH levels is assessed in this study with the use of a combination of chemical and microbiological analytical techniques.

2. Materials and methods

2.1. Operation of sequencing batch reactors (SBRs)

Four SBRs were operated in this study to explore the effect of pH on the competition between PAOs and GAOs. The 6 h cycles consisted of approximately a 2 h anaerobic period, a 3 h aerobic period and a 1 h settle/decant period. Synthetic wastewater (composition detailed below) was fed to each SBR during the first 5 min of the anaerobic period. The hydraulic retention time (HRT) of each reactor was 24 h, while the sludge retention time (SRT) was maintained at approximately 8 days. Nitrogen gas was bubbled into the reactor during the anaerobic period at a flowrate of around 0.5 L/min to maintain strict anaerobic conditions. In the aerobic period, the dissolved oxygen (DO) concentration was controlled at 3 ± 0.2 mg/L using an on/off control valve that was connected to a compressed air supply. The pH was controlled in each SBR during the anaerobic and aerobic phases using a one-way controller that dosed 0.5 M HC1 when the pH was above the setpoint. At times the pH dropped below the setpoint, particularly when that setpoint was 8 (see Table 1 for the pH setpoint

Table 1
The pH setpoint during each phase of SBR operation

SBR	Carbon source	pH setpoint
1—Phase I	Propionate	7
1—Phase II	Propionate	8
2—Phase I	Acetate	7
2—Phase II	Acetate	8
3	Propionate	8
4	Propionate	7

of each SBR). The pH setpoint therefore reflects the maximum pH observed at any time during the operation of each SBR.

The differences in the experimental conditions for the SBRs are highlighted in Table 1. SBR 1 and SBR 2 tested the long-term changes in the P removal performance and PAO/GAO populations resulting from an increase in the pH of the system from ~7 (phase I) to ~8 (phase II). The sole carbon source in the feed for SBRs 1 and 2 was propionate and acetate, respectively. The carbon source was varied in these reactors in order to select for different populations of bacteria, and thus assess the impact of an increase in pH on *Accumulibacter* PAOs, alphaproteobacterial GAOs and *Competibacter* GAOs. The seeding sludge (pH ~7) for SBRs 1 and 2 was obtained from a local EBPR plant in Queensland, Australia.

SBR 3 was operated to further test the competition between *Accumulibacter* PAOs and alphaproteobacterial GAOs at high pH (~8). SBR 3 was fed with propionate as the sole carbon source, and was seeded with a mixture of sludge (approximately 50% each) from SBR 1 (phase II) and an alphaproteobacterial GAO enriched sludge reported in Oehmen (2005).

Sludge from an additional reactor (SBR 4) reported by Oehmen et al. (2005c) was used in a series of batch tests at varying levels of pH (6.5–8.5) to test the immediate impact of a pH change on an enriched culture of PAOs. These batch tests were operated with 1.5 L of waste sludge from SBR 4, under otherwise identical conditions as outlined for SBR 1 apart from the pH. The carbon source for each test was propionate, while the pH was controlled at ±0.05 during these batch tests through addition of 0.05 M HCl and 0.05 M NaOH.

2.2. Synthetic media

The COD concentration in the feed for each SBR was 800 mg COD/L, while the phosphorus concentration was 53.3 mg PO_4-P/L, which yields a COD/P ratio of 15 mg COD/mg PO_4-P. The concentration of the other nutrients in the synthetic feed are indicated below (per litre): 0.11 g NH_4Cl, 0.05 g peptone, 0.17 g $MgSO_4 \cdot 7H_2O$, 0.08 g $CaCl_2 \cdot 2H_2O$, 2 mg allyl-*N* thiourea (ATU, a nitrification inhibitor), and 0.6 mL of a trace metals solution. The trace metals solution has also been described in Smolders et al. (1994) and consisted of (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 $Na_2MoO_4 \cdot 2H_2O$, 0.12 g $ZnSO_4 \cdot 7H_2O$, 0.15 g $CoCl_2 \cdot 6H_2O$ and 10 g EDTA.

2.3. Analytical procedures

Orthophosphate (PO_4-P) was analysed using a Lachat QuikChem8000 flow injection analyser (FIA). VFAs were measured using high performance liquid chromatography (HPLC) with a HPX-87H 300 mm × 7.8 mm, BioRad Aminex ion exclusion HPLC column operated at 65 °C. FIA and VFA samples were obtained through filtering mixed liquor from the SBR using 0.22 μm Millex GP syringe driven filters. Total suspended solids (TSS) and volatile suspended solids (VSS) were analysed according to standard methods (APHA, AWWA and WPCF, 1995).

Glycogen was determined via the modified method of Bond et al. (1999). Pre-weighed samples of lyophilised sludge were added to 5 mL of 0.6 M hydrochloric acid and digested for 6 h at 100 °C. After cooling, the samples were centrifuged and 1 mL of the supernatant liquid was analysed for glucose using HPLC. The column was also a HPX-87H 300 mm × 7.8 mm, BioRad Aminex ion exclusion HPLC column operated at 65 °C. The measured glucose equivalents were assumed to be derived from intracellular glycogen (Filipe et al., 2001a; Zeng et al., 2003). The PHA analytical method used in this study was described in detail in Oehmen et al. (2005a) for poly-β-hydroxybutyrate (PHB), poly-β-hydroxyvalerate (PHV), and poly-β-hydroxy-2-methylvalerate (PH2MV). In brief, pre-weighed samples of lyophilised sludge were added to 2 mL of chloroform and 2 mL of an acidified methanol solution (3–10% sulphuric acid by volume, approximately 100 mg/L of sodium benzoate). These samples were then digested for 20 h at 100 °C, cooled to room temperature, and 1 mL of water was added and mixed with each sample. One hour of settling time was allowed for phase separation. The chloroform phase was then extracted from the samples, mixed with ~1 g of sodium sulphate, and then separated from the solid phase. Three microliters of the chloroform phase was analysed by gas chromatography (GC) using a flame ionisation detector (FID) at 300 °C and a DB-5 non-polar capillary column (30 m × 0.25 mm × 0.25 μm) at a range of 80–270 °C.

2.4. Microbial analysis

FISH was performed as detailed in Amann (1995). The EUBMIX probe was used to target the entire

bacterial community (Daims et al., 1999), in combination with the PAO, GAO and *Alphaproteobacteria* FISH probes. The PAOMIX probe (Crocetti et al., 2000) was used to target *Accumulibacter,* a known PAO. The GAOQ989 probe (Crocetti et al., 2002) in conjunction with the GBG2 probe (Kong et al., 2002) were used to target *Competibacter,* a known GAO. The ALF969 probe (*E. coli* positions 986-969; 5′-TGGTAAGGTTC-TGCGCGT-3′) was used to target all *Alphaproteobacteria* (Crocetti et al., submitted for publication). This probe was modified from the previously developed ALF968 probe (Neef, 1997) to differentiate *Alphaproteobacteria* from *Competibacter,* a *Gammaproteobacteria* that is also targeted by the ALF968 probe. The optimal formamide concentration in the hybridisation buffer for ALF969 was determined by Crocetti et al. (submitted for publication) to be 35%. The SBR9-la probe was used to target a proposed alphaproteobacterial GAO (Beer et al., 2004), however, bacteria in this study did not bind to this probe. Since specific FISH probes to target the alphaproteobacterial GAOs are currently still being developed, their changes in population were not quantified in a similar manner as *Accumulibacter* and *Competibacter.* Their presence in these systems was assessed qualitatively through the use of the more generic ALF969 probe for all *Alphaproteobacteria.*

The probed sludge was examined using a Zeiss LSM510 Meta confocal laser-scanning microscope (Zeiss, Germany). Quantification of the PAO and GAO communities with respect to the entire bacterial population was determined via FISH image analysis using previously described methods (Bouchez et al., 2000; Crocetti et al., 2002). Thirty-five to fifty separate images were taken for this analysis, with the final results reflecting the mean percentages of *Accumulibacter* and *Competibacter* of the entire bacterial community by area. The standard error of the mean (SE_{mean}) was calculated as the standard deviation of the area percentages divided by the square root of the number of images analysed.

3. Results

3.1. PAO–GAO competition in a propionate-fed reactor (SBR 1)

In phase I, SBR 1 was operated with propionate as the carbon source and the pH controlled at 7. As shown in Fig. 1, a poor level of phosphorus removal was achieved during this period. A low amount of P release and uptake was associated with high levels of anaerobic propionate uptake, glycogen degradation and PHA accumulation (see Fig. 2A), which implies a high level of GAO activity during phase I. A summary of the chemical transformations and the *Accumulibacter* population during phase I is provided in Table 2. FISH analysis (see Fig. 3A) showed a large amount of *Alphaproteobacteria*, and a low *Accumulibacter* population, which correlates well with the high GAO activity observed chemically. On Day 21 the pH setpoint was increased from 7 to 8 (phase II), and after only a two-week period the phosphorus removal increased

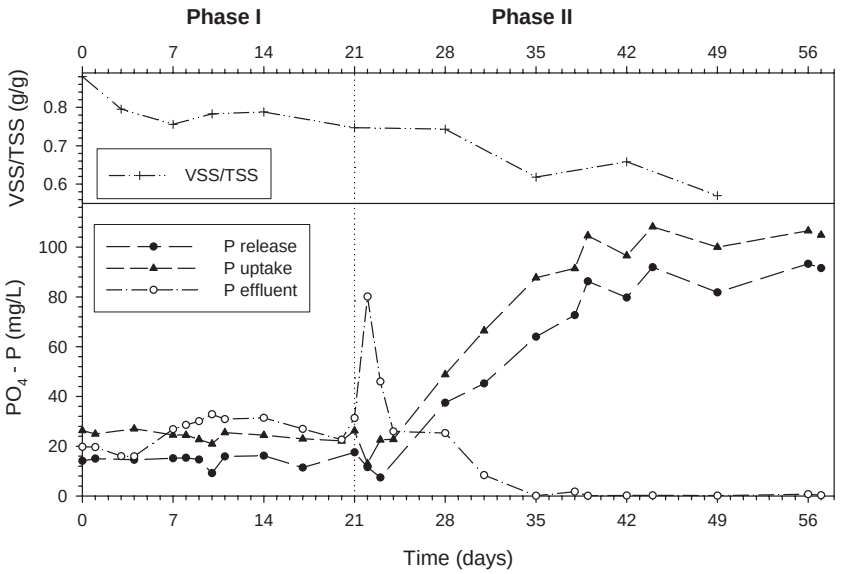

Fig. 1. Phosphorus removal performance for SBR 1 (propionate-fed) during the transition from phase I (pH = 7) to phase II (pH = 8).

substantially (Fig. 1). A high level of phosphorus removal was achieved throughout the remainder of phase II at elevated pH.

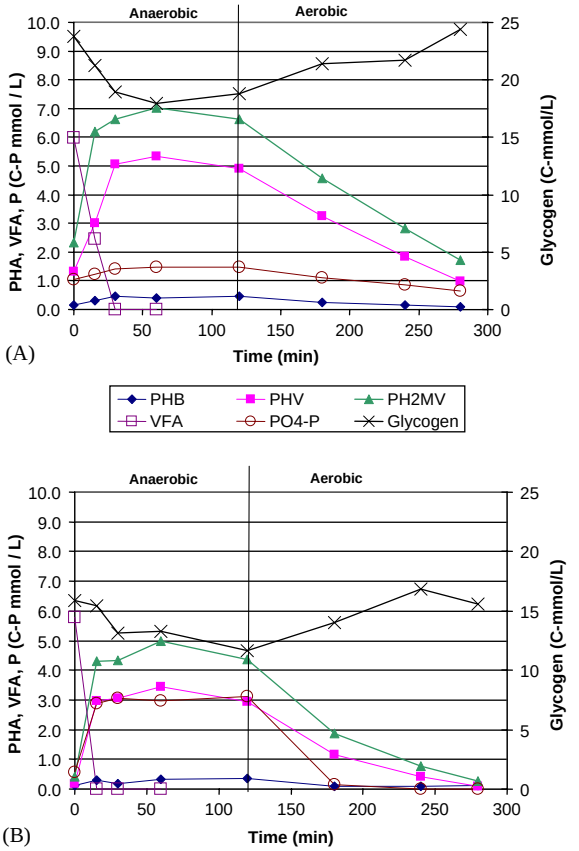

Fig. 2. Cycle studies during phase I (A) and phase II (B) of SBR 1 operation. A transition occurred between the GAO (A) and PAO (B) phenotypes.

The carbon and phosphorus transformations during a cycle of SBR 1 (phase II) is shown in Fig. 2B, while the chemical characteristics and *Accumulibacter* population during this period are summarised in Table 2. The glycogen degradation and PHA transformations were lower in phase II than phase I, suggesting a reduction in the GAO activity after the pH was increased. The lower VSS/TSS ratio implied that there was a higher level of phosphorus stored in the sludge in phase II (Fig. 1), which suggested that there was an increase in the number of PAOs. This is supported by the FISH results, which show an increase in the *Accumulibacter* population (Table 2). The higher PAO activity during phase II as compared with phase I is further reflected through the increase in the P release to VFA uptake ratio. Therefore, the two-week transition period between low and high P removal observed in SBR 1 was very well explained by a population shift from GAOs to PAOs.

In the first day of operation following the pH increase of SBR 1, the effluent phosphorus concentration increased to a level higher than the P concentration in the feed (53.3 mg PO_4-P/L). The reason for this is presently unclear. The sudden change in pH could have triggered a disruption to the metabolism of the PAOs, or perhaps there was an external disturbance that could not be explained. The quick recovery in P removal exhibited by SBR 1 suggests that this disturbance was only a temporary phenomenon.

Previous studies have shown that a propionate feed source often produces improved EBPR operation as compared to acetate (Thomas et al., 2003; Chen et al., 2004; Oehmen 2005), likely due to the inhibition of GAOs, particularly *Competibacter* (Pijuan et al., 2004; Oehmen 2005; Oehmen et al., 2005b). Alphaproteobacterial GAOs can be enriched with propionate as the carbon source, however, and SBR 1 (phase I) suggests

Table 2
SBR 1 (propionate-fed) and SBR 2 (acetate-fed): summary of the anaerobic chemical transformations and FISH quantification

	SBR1		SBR 2		Units
	Phase I	Phase II	Phase I	Phase II	
P/VFA	0.08	0.44	0.10	0.36	P-mol/C-mol
Glycogen/VFA	0.81	0.48	1.18	0.85	C-mol/C-mol
PHB/VFA	0.05	0.04	1.13	1.13	C-mol/C-mol
PHV/VFA	0.60	0.48	0.49	0.37	C-mol/C-mol
PH2MV/VFA	0.72	0.68	0.00	0.01	C-mol/C-mol
PHA/VFA	1.37	1.20	1.62	1.51	C-mol/C-mol
Accumulibacter	8	33	14	15	%
SE_{mean}	1	2	2	2	%
Competibacter	<1	<1	54	23	%
SE_{mean}	—	—	4	1	%

Notes: The alphaproteobacterial GAOs were not quantified, because specific FISH probes to target this organism have not yet been developed. The SBR 1 samples were obtained on Day 1 (Phase I) and Day 42 (Phase II), respectively. The SBR 2 samples were obtained on Day 7 (Phase I) and Day 112 (Phase II), respectively. See nomenclature for abbreviations.

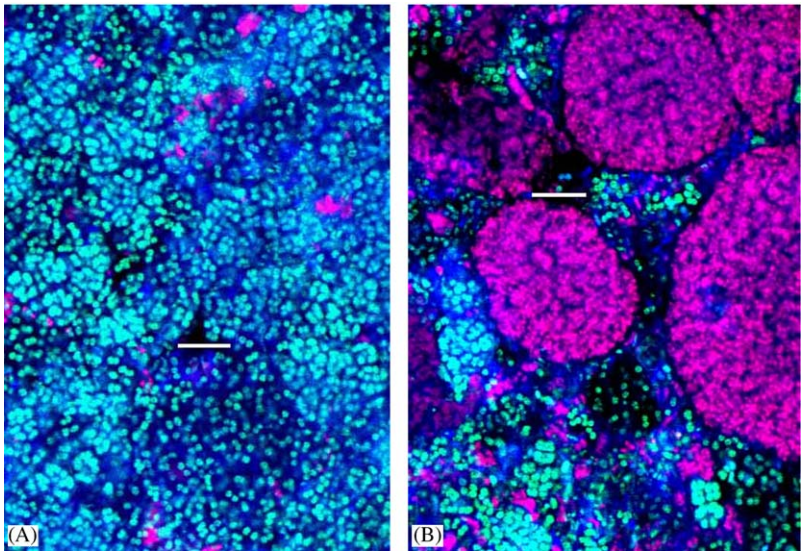

Fig. 3. FISH images from SBR 1 phase I (A) and phase II (B). *Accumulibacter* are shown in pink, *Alphaproteobacteria* are shown in cyan and all other bacteria are shown in blue (Bar = 10 μm). An increase in the pH from ~7 to ~8 resulted in an increase in the *Accumulibacter* population and a decrease in the number of *Alphaproteobacteria* from phase I to phase II. The alphaproteobacterial GAOs are likely to exhibit a tetrad morphology, and appeared to decrease in abundance from phase I to phase II.

that these GAOs may indeed compete effectively with PAOs for propionate uptake in certain cases. As shown in the results from SBR 1 (phase II), PAOs appear to be much more efficient at a high pH than the so-called alphaproteobacterial GAOs, and were able to increase in number after the pH was increased (see Fig. 3B). These results suggest that a high pH may be an effective means of maximising PAO activity and minimising the proliferation of alphaproteobacterial GAOs in EBPR systems.

3.2. PAO–GAO competition in the acetate-fed reactor (SBR 2)

SBR 2 was operated using acetate as the carbon source in order to compare the effect of pH on the microbial competition between *Accumulibacter* PAOs and *Competibacter* GAOs. The phosphorus removal performance from SBR 2 is shown in Fig. 4. The pH was controlled at 7 during phase I of operation, and during this period the P removal was consistently observed to be very low, with an effluent P concentration that was even higher than was observed during phase I of SBR 1. A cycle study during phase I (Fig. 5A) reveals the GAO phenotype of anaerobic VFA uptake, glycogen hydrolysis and PHA accumulation, coupled with aerobic PHA oxidation and glycogen replenishment. FISH analysis showed that the dominant microorganism was indeed *Competibacter* (Fig. 6A). On Day 42, the pH was increased from 7 to 8 (phase II). In this case, quasi-steady state of the P removal in the reactor was not achieved until approximately 4 weeks after the increase in pH (Fig. 4). During this period, the P release and uptake transformations gradually increased, while the effluent phosphorus concentration decreased. Unlike SBR 1, however, the effluent phosphorus concentration did not reach zero after quasi-steady-state operation resumed.

Fig. 5B shows a cycle during the quasi-steady-state operation of phase II, while the abundance and activity of PAOs and GAOs in phases I and II of the operation are compared in Table 2. The number of *Accumulibacter* is very similar in phase II as compared to phase I, but the *Competibacter* population decreased substantially (see Fig. 6B). The level of glycogen hydrolysis is clearly reduced from phase I to phase II, as is the level of PHV accumulation (see Table 2). According to the proposed PAO and GAO metabolism, GAOs tend to produce more PHV than PAOs when fed with acetate, due to partial glycogen hydrolysis through the succinate–propionate pathway (Filipe et al., 2001a). Therefore, the reduced levels of glycogen hydrolysis and PHV accumulation in phase II suggest a reduction in GAO activity, which correlates very well with the lower *Competibacter* population. Similarly, the increase in the P release/VFA uptake ratio and reduction of the VSS/TSS ratio suggest an increase in the level of PAO activity. This is likely due to the decreased *Competibacter* population as well, since *Accumulibacter* would have less competition with GAOs for acetate uptake. This hypothesis is supported by the lower total acetate uptake rate observed during phase II (Fig. 5B) as compared to

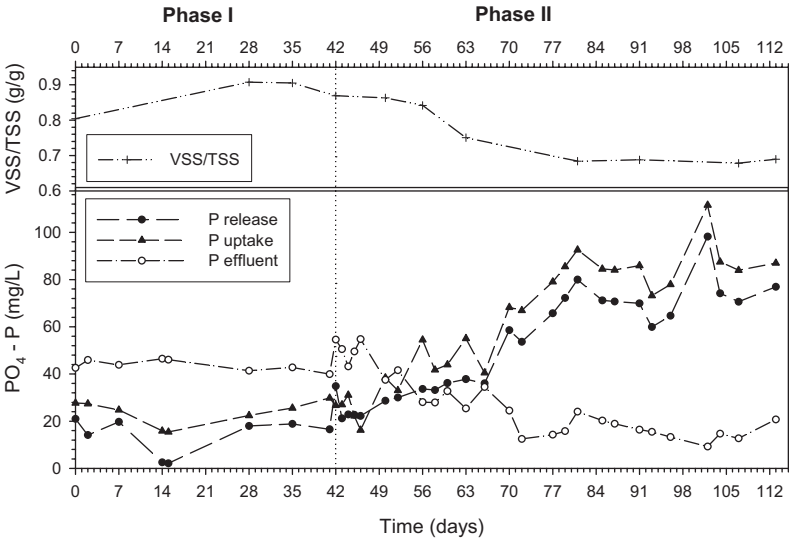

Fig. 4. Phosphorus removal performance for SBR 2 (acetate-fed) during the transition from phase I (pH = 7) to phase II (pH = 8).

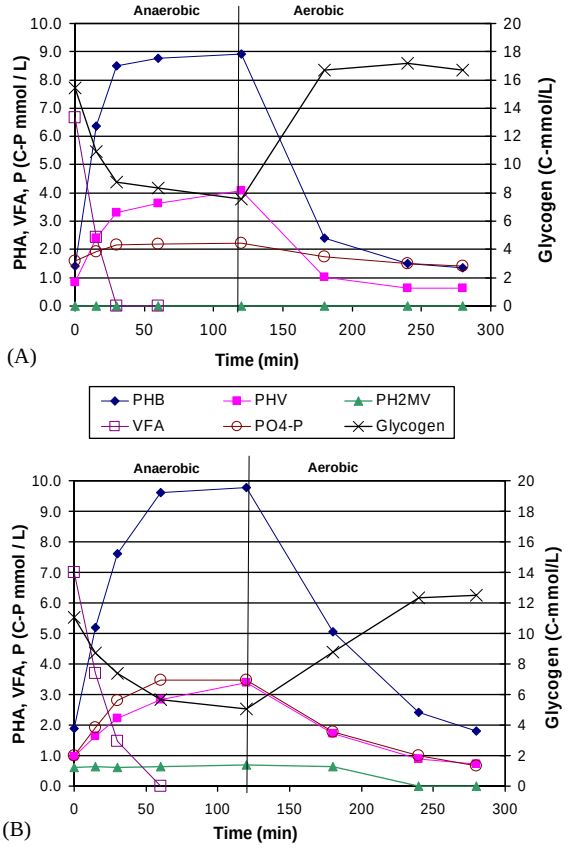

Fig. 5. Cycle studies during phase I (A) and phase II (B) of SBR 2 operation.

phase I (Fig. 5A). A decrease in the acetate uptake rate suggests that the total number of PAOs and/or GAOs decreased, which correlates well with the reduction in *Competibacter* found using FISH.

The results from SBR 2 strongly suggest that a high operating pH provided PAOs an advantage over GAOs. However, there was a considerable number of *Competibacter* remaining at high pH, while the *Accumulibacter* population did not increase substantially. The results from SBRs 1 and 2 suggest that PAOs compete more effectively with alphaproteobacterial GAOs for propionate uptake than with *Competibacter* for acetate uptake. This supports the hypothesis that propionate is a better carbon source for reliable EBPR operation than acetate, as has been suggested previously by Oehmen (2005).

3.3. PAO–GAO competition in a mixed culture fed with propionate (SBR 3)

In order to further test the impact of a high pH on the competition between *Accumulibacter* PAOs and alphaproteobacterial GAOs, SBR 3 was seeded with a mixture of PAOs and GAOs, and was operated with a pH setpoint of 8. The phosphorus removal performance of SBR 3 is shown in Fig. 7, while Fig. 8 shows a cycle study from Day 43. A stable level of P removal was achieved throughout the study. A rapid increase was observed in the P release and P uptake transformations during the initial start-up period of 7–10 days, suggesting that the PAO activity increased relative to the activity by GAOs. The reduction of the VSS/TSS ratio

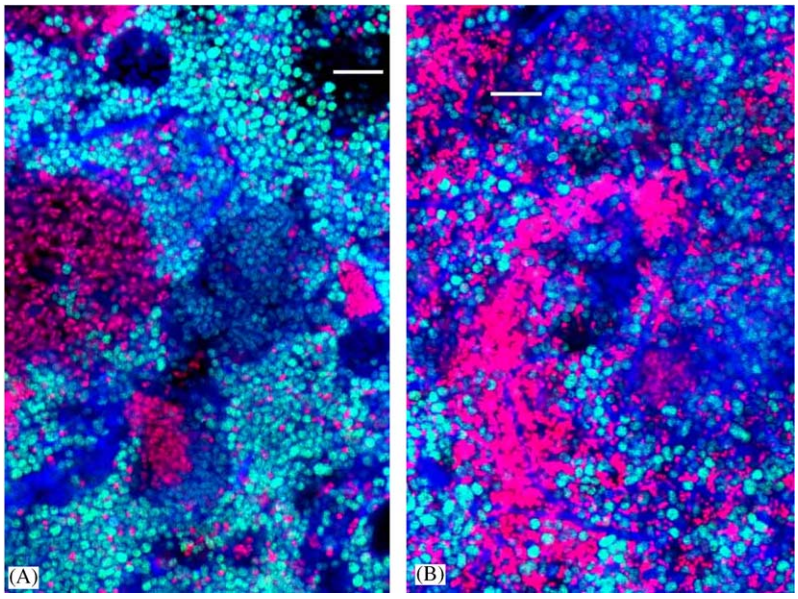

Fig. 6. FISH images from SBR 2 phase I (A) and phase II (B). *Accumulibacter* are shown in pink, *Competibacter* are shown in cyan and all other bacteria are shown in blue (Bar = 10 μm). An increase in the pH from ~7 to ~8 resulted in a decrease in the *Competibacter* population from phase I to phase II.

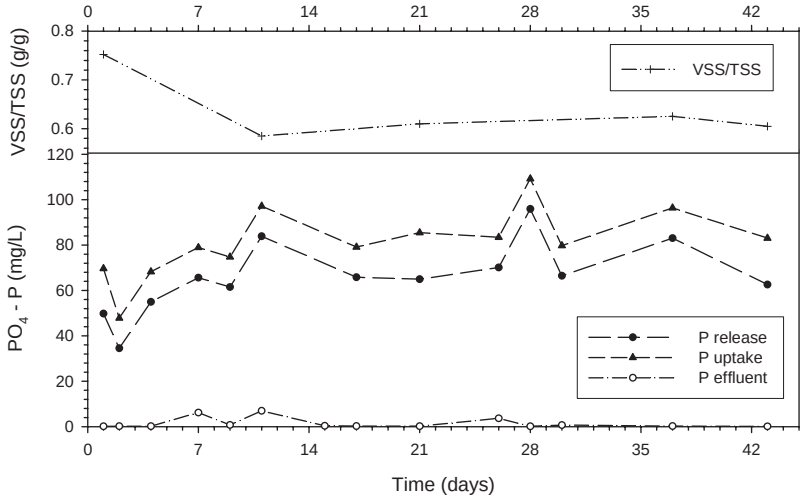

Fig. 7. Phosphorus removal performance during operation of SBR 3 (pH = 8).

over the start-up period implies that a higher level of phosphorus was stored in the sludge, and suggests an increase in the PAO population. These findings are supported by the FISH analysis, which is shown in Figs. 9A (Day 1) and 9B (Day 43). The *Accumulibacter* population rose from 22% ($SE_{mean} = 2\%$) to 51% ($SE_{mean} = 2\%$) during this time, while the number of *Alphaproteobacteria* was observed to decrease, suggesting that the GAO population also decreased. Therefore, the PAOs again appeared to have an advantage over the alphaproteobacterial GAOs at high pH, in a similar fashion as shown in SBR 1. The results from SBRs 1–3 indicate that a high pH is indeed beneficial for a high level of phosphorus removal in EBPR systems, due to the selection of PAOs over GAOs.

3.4. Short-term effects of pH on PAOs fed with propionate as the sole carbon source

A PAO enriched sludge fed with propionate as the sole carbon source (SBR 4) was operated over a 2 month period as is described in Oehmen et al. (2005c).

Anaerobic–aerobic batch tests were performed using sludge from SBR 4 at pH values ranging from 6.5 to 8.5. The results from these tests are summarised in Table 3. There was a higher P release to VFA uptake ratio and a lower glycogen degradation observed at high pH (8.0 and 8.5) as compared with low pH. This suggests that either there was a reduced level of GAO activity at these pH levels, or that PAOs tend to rely more on polyphosphate hydrolysis and less on the glycolysis of glycogen as the energy source for propionate uptake at high pH. At pH values of 7.5 and 8.0, there was an increase in the rate of VFA uptake, phosphorus uptake and biomass growth (shown as the rate of ammonia uptake, since nitrification was inhibited in the SBR by the presence of ATU) as compared with the other values of pH tested. These results show that both the anaerobic and aerobic PAO activity was enhanced at high pH values (i.e. 7.5 and 8.0), and suggests that a high pH is more beneficial for PAOs than a low pH. This supports the findings from the other propionate-fed SBRs, where an increase in the level of PAO activity was also observed at high pH.

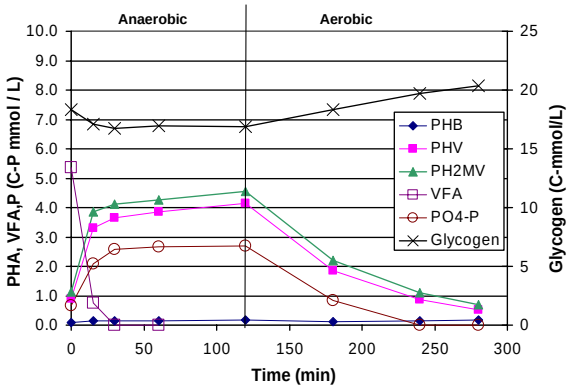

Fig. 8. A cycle of SBR 3 on Day 43, clearly demonstrating the PAO phenotype.

4. Discussion

The results from this study showed that phosphorus removal performance improved after the pH was increased from 7 to 8, due to population shifts favouring PAOs over GAOs, which was shown through both chemical and microbiological methods. Thus, it is proposed that EBPR operation at a high pH level (~8) can potentially reduce the proliferation of GAOs and lead to improved phosphorus removal efficiency in EBPR systems. The results suggest that full-scale wastewater treatment plants suffering from poor phosphorus removal could achieve greater phosphorus removal performance and stability from increasing the pH of their system. The implementation of this control strategy in a full- or pilot-scale study would be of interest to evaluate cost-effectiveness and confirm that superior process performance is achieved through this approach.

Propionate-fed reactors at high pH (i.e. SBR 1 phase II and SBR 3) showed a higher level of P removal than an acetate-fed reactor (SBR 2) at a similar pH. Although PAOs were observed in this study to gain an advantage at high pH over GAOs in both systems, the results suggested that PAOs possessed a greater advantage over the alphaproteobacterial GAOs than *Competibacter*. This could be due to an ability by PAOs to metabolise propionate at a faster rate than acetate when the pH is high. A recent study by Oehmen et al. (2005c) showed that the maximum rate of propionate uptake by an enriched culture of PAOs at a pH of 7 was similar to the

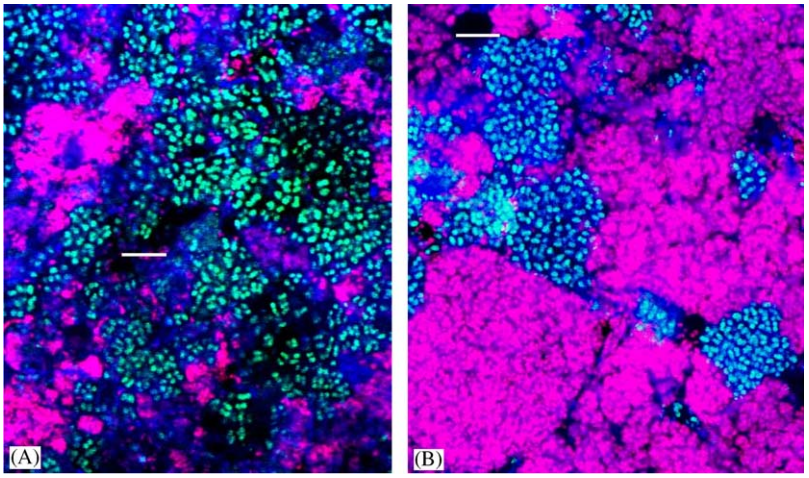

Fig. 9. FISH images of SBR 3 on Day 1 (A) and Day 43 (B). *Accumulibacter* are shown in pink, *Alphaproteobacteria* are shown in cyan and all other bacteria are shown in blue (Bar = 10 μm). Once again, *Accumulibacter* increased in abundance while the *Alphaproteobacteria* population appeared to decrease at elevated pH (~8).

Table 3
Stoichiometric and kinetic metabolism by a propionate enriched PAO reactor (SBR 4) during a short-term change in pH

pH	6.5	7.0	7.5	8.0	8.5	Units
VFA uptake rate	191	175	276	248	233	mg COD/g VSS-h
PO_4-P release rate	61.5	56.3	93.2	103.8	88.8	mg PO_4-P/g VSS-h
PO_4-P uptake rate	23.9	25.6	37.7	36.2	27.3	mg PO_4-P/g VSS-h
NH_4-N uptake rate	0.63	0.96	1.13	1.01	0.70	mg NH_4-N/g VSS-h
P/VFA	0.39	0.39	0.41	0.51	0.46	P-mol/C-mol
Glycogen/VFA	0.36	0.32	0.25	0.12	0.15	C-mol/C-mol
PHB/VFA	0.04	0.04	0.05	0.04	0.00	C-mol/C-mol
PHV/VFA	0.55	0.55	0.45	0.34	0.41	C-mol/C-mol
PH2MV/VFA	0.65	0.65	0.61	0.58	0.60	C-mol/C-mol
PHA/VFA	1.24	1.23	1.10	0.95	1.01	C-mol/C-mol

Note: See nomenclature for abbreviations.

maximum acetate uptake rate from enriched PAO cultures in literature studies at the same pH. The acetate uptake rate of enriched PAO reactors has been shown in batch testing to be independent of pH over the range of 6.5–8.0 (Smolders et al., 1994; Liu et al., 1996; Filipe et al., 2001c). In Table 3, however, it can be observed that the propionate uptake rate by an enriched PAO reactor was faster at high pH (i.e. 7.5–8.0) than at low pH (i.e. 6.5–7.0). The total anaerobic VFA uptake rate in the propionate-fed SBR 1 was faster at high pH (Fig. 2B) than at low pH (Fig. 2A), while the reverse was true in the acetate-fed SBR 2 (Fig. 5), which further supports this hypothesis. A higher uptake rate of propionate than acetate by PAOs at elevated pH levels could explain why the alphaproteobacterial GAOs appeared to be out-competed more rapidly than *Competibacter* GAOs at high pH.

Further investigations are necessary to explore the metabolic mechanism by which PAOs are provided a selective advantage over GAOs in EBPR systems at high pH, as has been shown in this study.

5. Conclusions

The results of this study show that a high operational pH repeatedly stimulated an increased level of phosphorus removal in EBPR systems, through providing PAOs an advantage over their competitors, the GAOs. The results also suggest that PAOs may compete more effectively with alphaproteobacterial GAOs for propionate uptake than with *Competibacter* for acetate uptake. A higher level of phosphorus removal was achieved in propionate-fed systems as compared to an acetate-fed system, supporting previous study showing that propionate was a better carbon source for reliable EBPR operation than acetate. Implementation of control strategies to favour a high operational pH in full-scale EBPR wastewater plants may lead to improvements in phosphorus removal performance.

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