Phosphorus release and uptake during start-up of a covered and non-aerated sequencing batch reactor with separate feeding of VFA and sulfate

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ABSTRACT

This study explored a sulfur cycle-associated biological phosphorus (P) removal process in a covered and non-aerated sequencing batch reactor (SBR) fed with volatile fatty acid (VFA) and sulfate separately. During the 60-day start-up, both phosphate release and uptake rates increased, while poly-phosphate cyclically increased and decreased accordingly. The P-release and P-uptake rates were associated with VFA uptake and sulfate reduction. The average ratio of potassium to phosphate during the P-uptake and P-release was also determined to be 0.29–0.31 mol K/mol P, which is close to a reported value (0.33) for biological phosphorus removal. All this evidence confirmed there was biological P removal in this reactor, in which metabolism could be different from conventional biological P removal.

Key words | phosphate release and uptake, sequencing batch reactor, sulfur cycle-associated process

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INTRODUCTION

Hong Kong has practised seawater toilet flushing since the 1950s to solve water scarcity problems (Tang *et al*. 2006). As a reliable, economic and sustainable alternative water resource, the saline water supply saved 20% of valuable fresh water for 80% of the inhabitants (WSD 2009). However it resulted in saline sewage containing 500 mg/L sulfate on average originating from seawater. Although corrosion and odor generation in pressurized sewers can be controlled cost-effectively (DSD 2011), hydrogen sulfide emission during primary sewage treatment and subsequent anaerobic sludge treatment needs to be controlled.

In order to maximize the benefits of the dual water supply system we have recently developed a novel sulfur (S) cycle-based biological nitrogen removal process, named the Sulfate reduction Autotrophic denitrification Nitrification Integrated (SANI®) process (Lau *et al*. 2006; Wang *et al*. 2009; Lu *et al*. 2011). The SANI process applies biological sulfate reduction to remove COD in an anaerobic bioreactor, thereby providing sufficient electron donors (sulfide) for subsequent autotrophic denitrification (Lu *et al*. 2009). Both sulfate-reducing bacteria (SRB) and autotrophic bacteria for nitrification and denitrification have low biomass yields, therefore producing minimal sludge and no odor (Lu *et al*.

2011). The SANI process provides an energy-saving solution for saline sewage treatment in Hong Kong.

The current SANI process removes organic matter and nitrogen only. However, phosphorus has to be removed from saline sewage in the near future, due to the new restriction on the standard of discharge. Since several organisms have the ability to accumulate poly-phosphates (van Loosdrecht *et al*. 1997; Oehmen *et al*. 2007), we attempted to investigate the possibility of enhanced biological phosphorus removal (EBPR) associated with the sulfur cycle. The purpose of this study is therefore to confirm the existence of EBPR in an extended SANI process. For this purpose, we applied a covered and non-aerated sequencing batch reactor (SBR) with separate feeding of volatile fatty acid (VFA) and sulfate to induce a sulfur cycle-associated EBPR.

MATERIALS AND METHODS

SBR and its operation

A 20-L SBR was operated in a constant-temperature chamber $(22 \pm 1\,°C)$ following the operation scheme

shown in Figure 1. The reactor was seeded with sludge taken from a local secondary saline sewage treatment works. Ten litres of synthetic wastewater was fed to the reactor within 0.25 h in each cycle. The total cycle time was up to 141 h per cycle, and decreased to about 100 h per cycle from the start-up to after 60 days. Synthetic wastewater composition was modified from Kuba *et al.* (1993) in terms of organic carbon and phosphorus concentrations, which contained 400 mg/L COD and 20 mg P/L. It was prepared from 0.521 g/L NaAc, 0.067 g/L K_2HPO_4 and 0.035 g/L KH_2PO_4. Appropriate amounts of nitrogen and macro-minerals were added to the feed by adding 0.50 g/L $MgCl_2.6H_2O$, 0.07 g/L $CaCl_2.H_2O$, 0.227 g/L NH_4Cl, and 0.01 g/L EDTA (Kuba *et al.* 1993), and 2.0 ml/L trace mineral solution (Lau *et al.* 2006). Sulfate prepared from sodium sulfate was fed at 10 mg S/L. The reactor was mixed continuously by a mixer at a rotation speed of 150 rpm. It was completely covered throughout the study.

Phase I was controlled manually until VFA-uptake and P-release were completed, i.e. all VFAs were consumed with no more P released. The pH in Phase I was controlled at 7.3 ± 0.5. In Phase II, sulfate was dosed. Phase II lasted until no more P uptake was observed, followed by settling and decanting. After 1 h-settling, 10 L of supernatant was decanted as the effluent. Oxidation Reduction Potential (ORP) of the reactor was monitored throughout Phases I and II, which generally decreased to −300 mV in Phase I and increased to −200 mV in Phase II.

Analytical method

Concentrations of phosphate, sulfide, mixed liquor suspended solid (MLSS) and mixed liquor volatile suspended solid (MLVSS), and phosphorus in sludge were analyzed regularly according to the Standard Methods (APHA 1998). Alkalinity was measured using a 5-point pH titration method (Moosbrugger *et al.* 1993). Sulfate and acetate (VFA) were analyzed using a liquid-chromatograph (Shimadzu). Ammonia was determined from a Flow Injection Analyzer (Quickchem FIA+). Potassium (K^+) was measured using an Atomic Adsorption Spectrophotometer (VARIAN 220FS). Poly-phosphate in the sludge was visualized with DAPI (4′,6′-diamidino-2-phenyl indol dihydrochloride) staining using a Zeiss Laser Scanning Confocal Microscope (Model: LSM7 DUO (710 + Live)) (Serafim *et al.* 2002). To quantify the phosphate fractions of the sludge, we adopted a sequential extraction method (Uhlmann *et al.* 1990).

RESULTS AND DISCUSSION

Performance during the system start-up

During the 20 cycles or about 60 days, the average biomass concentrations were about 2,300 mg VSS/L, during which no sludge was purposely withdrawn from the reactor. Based on the solids loss via the effluent, the solids retention time (SRT) and observed sludge yield coefficient during this start-up period were determined to be 100 days and 0.04 g VSS/g COD, respectively. Figure 2 shows that the P-release and P-uptake increased to 13 and 16 mg P/L in the period with a maximum P-uptake of up to 20 mg P/L.

Figure 3 shows the molar ratio of P-release to VFA consumption (ΔP-release/ΔVFA) in Phase I and that of P-uptake to sulfur change (ΔP-uptake/ΔS) in Phase II. The first ratio increased from 0 to 0.10 (mmol P/mmol C) during these 20 cycles, while the second ratio increased

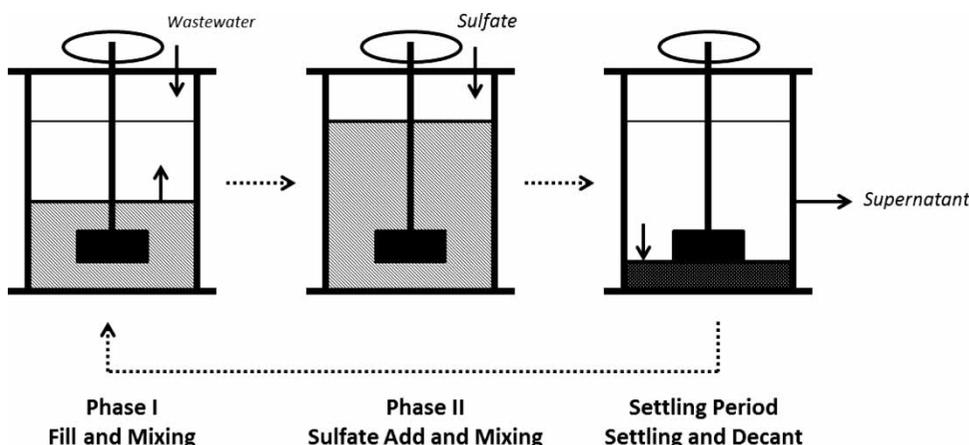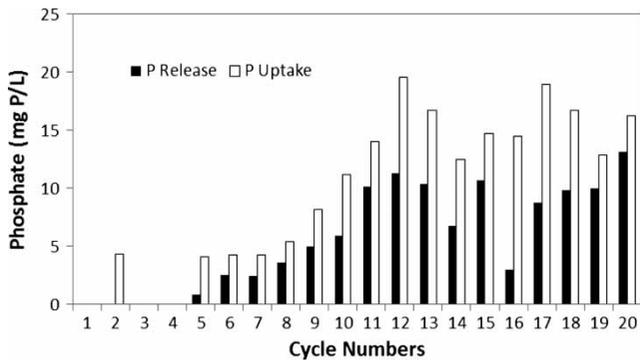**Figure 1** | Schematic diagram of the SBR operation phases.

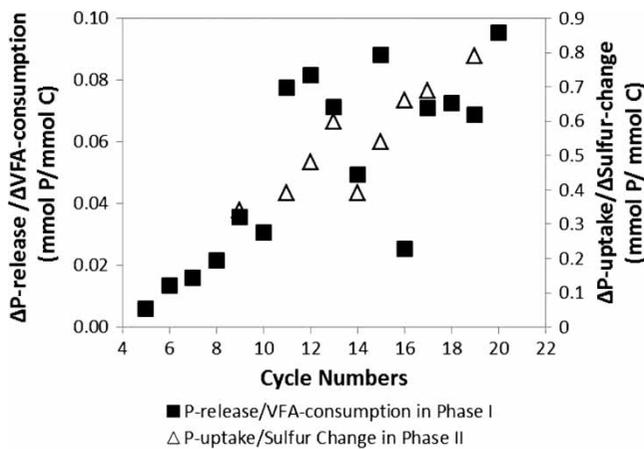**Figure 2** | P-release and P-uptake in SBR.

Figure 3 | P-release and VFA uptake in Phase I; P-uptake and sulfate change in Phase II.

from 0.34 to 0.79 (mmol P/mmol S) from Cycles 10 to 20. Both increasing trends were close to each other. This implied that all carbon, phosphorus and sulfur behaviors

could be well correlated, indicating that the sulfur cycle played a role in the P removal process.

Phosphorus balance

The phosphorus balance analysis was conducted in order to confirm phosphorus accumulation in the sludge due to EBPR. Figure 4 shows that the P content in the sludge increased by 2.5% within 60 days. The accumulative amount of phosphorus uptaken from the bulk liquid was 93.6 mg P/L, close to that in the sludge at 91.8 mg/L (from 41.8 initially to 133.6 mg P/L on Day 60) during this start-up period. These figures confirmed the removed phosphorus was almost completely stored in the sludge.

Confirmation of biological phosphorus removal

Table 1 shows the mean concentration variations of potassium and phosphate in Phase I and Phase II (Cycles 17–20). It was found that the molar ratio of potassium variation (K^+-release) to P-release (P-release) (K^+-release/P-release) in Phase I was determined to be 0.31 and that of K^+-uptake to P-uptake in Phase II was 0.29 (mmol K/mmol P), indicating that EBPR might occur in this SBR reactor. In order to confirm this, a sequential extraction was conducted to quantify the phosphate fractions in the sludge of Cycle 20 (Uhlmann *et al.* 1990). This test found that nearly 91% of phosphates was organic bound phosphate (mainly polyphosphate), and the remaining 9% of phosphates was composed of adsorbed or bounded phosphorus. This indirectly
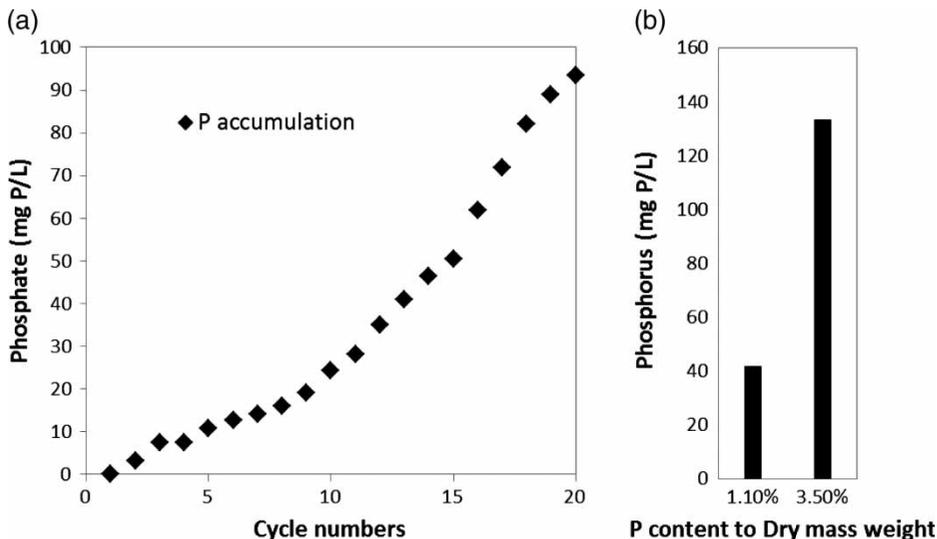

Figure 4 | (a) Phosphate removed from bulk liquid and (b) phosphorus content in sludge.
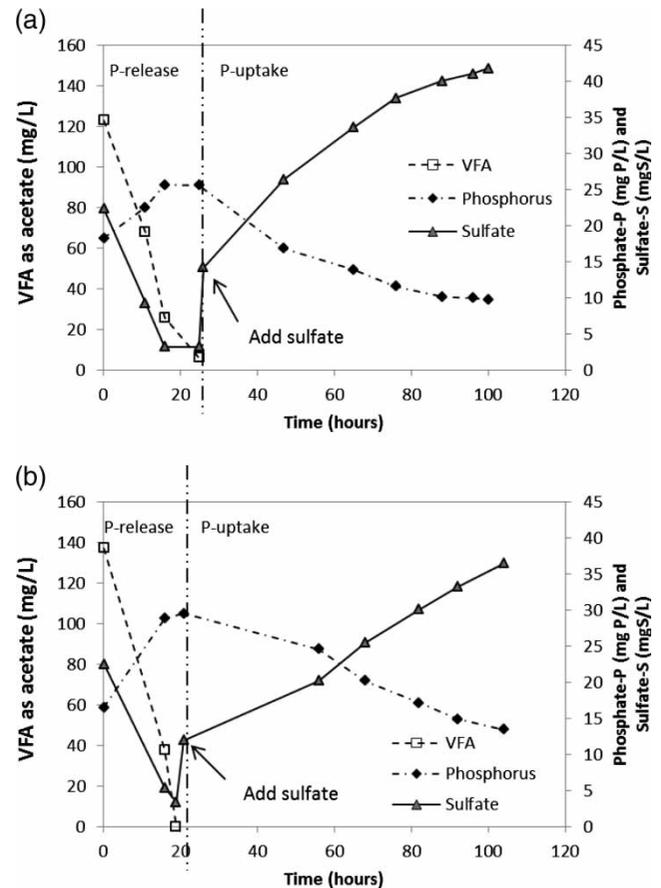
Table 1 | Variation of potassium and phosphate in Phase I and II

Phase	K⁺ variation	P variation	K^+/P mole ratio
I	K^+ release = 3.86 ± 0.39 (mg/L)	P release = 10.02 ± 1.87 (mg/L)	0.31
II	K^+ uptake = 6.03 ± 0.76 (mg/L)	P uptake = 16.05 ± 2.51 (mg/L)	0.29

**Figure 6** | Typical P-release and P-uptake (a) Cycle 17 and (b) Cycle 20.

confirmed that the observed P removal in this covered and non-aerated SBR with separate feeding of organics and sulfate was potentially biological.

Poly-phosphate (poly-P) in the reactor sludge was analyzed on Day 60, as shown in Figure 5. White spots reflected poly-P, their intensity was clearly lower in Phase I than that in Phase II. This is because poly-P degraded to release phosphate in Phase I while in Phase II poly-P formed via P-uptake. This confirmed that EBPR most likely occurred in the reactor.

Figure 6 shows typical variations of VFAs, PO₄, and sulfate concentrations during the P-release and P-uptake Cycle 17 (a) and Cycle 20 (b). Sulfate in the P-release of both cycles decreased from 22 to 3 mg S/L, and VFA was consumed almost completely, releasing 9 and 13 mg P/L, respectively. Comparatively 18.9 and 16 mg P/L were uptaken during the P-uptake, while sulfate remained increasing, in both cycles. VFA was possibly utilized separately by biological sulfate reduction and stored as poly-β-hydroxyalbutyrates (PHBs) to drive the P-release. Sulfur might be stored as elemental sulfur (S^0) and/or polysulfide (S_n^{2-}) (Fuseler & Cypionka 1995) within the sulfur-related Phosphorus Accumulating Organisms (S-PAOs) during the P-release. The observed P-uptake might be associated with degradation of PHAs and possible oxidation of stored

sulfur substance into sulfate, because an increase in the sulfate level at the end of P-uptake was observed. The sulfur oxidation is being studied through batch testing under anaerobic conditions. Although sulfate cannot be utilized as an electron acceptor in this phase, it might be in favor of the formation of poly-P. We further found that the
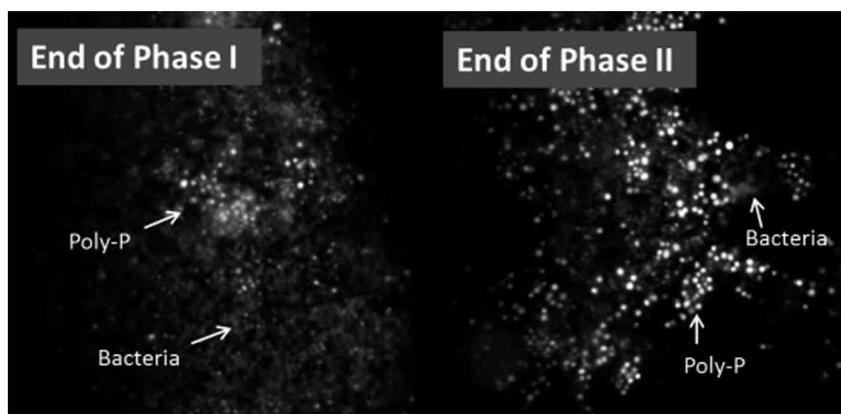
Figure 5 | Poly-phosphate (Poly-P) observed on Day 60 (grey points indicating bacteria and bright points indicating poly-phosphate).

amount of sulfate-S reduced in the P-release was equal to that of sulfide-S oxidized in the P-uptake. This could be attributed to an unknown EBPR metabolism. We are currently working on this through analysis of electron, charge and energy balances as well as the microbiological community.

CONCLUSION

This study explored a possibly new enhanced biological phosphorus removal process associated with a sulfur cycle in a covered and non-aerated SBR with separate feeding of VFA and sulfate. The main findings can be summarized below:

1. The P-release and P-uptake were increasing during the 60-day start-up period. Carbon, sulfur and phosphorus behaviors were correlated well during the P-release and P-uptake. Enhanced biological phosphorus removal most likely occurred in this SBR reactor, resulting in an opportunity of expending the SANI process into an EBPR application.
2. The mechanism of this EBPR could be associated with the sulfur cycle, which is new and deserves further study.

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