Simultaneous nitrification, denitrification and phosphorus recovery (SNDPr) - An opportunity to facilitate full-scale recovery of phosphorus from municipal wastewater

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Highlights

- Carbon in wastewater is adequate for nitrogen (N) removal and phosphorus (P) recovery
- Simultaneous nitrification denitrification & P removal (SNDPR) enables P recovery
- Dissolved oxygen < 0.5 mg/L critical for a balanced microbial community in granules
- Polyphosphate accumulating organisms showed highest affinity towards oxygen
- Glycogen accumulating organisms played a role in SNDPR, specifically to remove N

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Abstract

Sewage treatment plants are a potential point source for recycling of phosphorus (P). Several technologies have been proposed to biologically recover P from wastewater. The majority of these technologies are side-stream processes and rely on an external source of soluble organic carbon to facilitate P recovery. To date, no studies have demonstrated the potential to facilitate main-stream recovery of P, using carbon that is naturally present in wastewater. Simultaneous nitrification, denitrification and phosphorus removal (SNDPR) is an elegant process that can uptake influent carbon and effectively remove both nitrogen (N) and P from wastewater. SNDPR studies to date, however, have failed to facilitate a P rich liquor end-of-anaerobic-phase, that enables economies of scale to recover influent P. Therefore, this study examined the feasibility of achieving a P rich liquor (e.g. > 70 mg-P/L) with granular SNDPR process. A synthetic influent that replicated the nutrient and carbon concentrations of municipal wastewater was used to investigate whether carbon in the influent wastewater could enable both nutrient removal and P recovery from wastewater. Our granular SNDPR process was able to facilitate a P rich liquor of approximately 100 mg-P/L end-of-anaerobic-phase. A dissolved oxygen (DO) concentration of 0.5 mg/L in a sequencing batch reactor (SBR) was found to be essential to achieve complete nutrient removal and a high P concentration at the end of the anaerobic phase. At this steady state of reactor operation, the abundance of polyphosphate accumulating organisms (PAOs) was 2.6 times the abundance of glycogen accumulating organisms (GAOs). The study also demonstrated the importance of denitrifying polyphosphate accumulating organisms (DPAOs) and glycogen accumulating organisms (DGAOs) to achieve complete removal of N from the effluent. Compared to nitrifying bacteria, the polyphosphate accumulating organisms (PAOs) had a higher affinity towards DO. This study, for the first time, showed that the mainstream recovery of P is feasible using a SNDPR process.

Keywords: Phosphorus recovery, Polyphosphate accumulating organisms (PAOs), Denitrifying polyphosphate accumulating organisms (DPAOs), Simultaneous nitrification, denitrification and phosphorus removal (SNDPR).

1. Introduction

Modern agricultural practices are highly reliant on phosphorus (P) to achieve high crop yields. P however, is a non-renewable resource and depletion of P reserves is likely in the next 50 – 100 years (Shu et al., 2006). With the aim of reducing pressures on mining, there has been considerable interest on processes for P recycling in the recent past. Municipal wastewater treatment plants are a key point source for recycling of P (Cordell et al., 2009). However, the economics of P-recovery from this source are not encouraging due to the low concentrations typically found in influent wastewater (< 10 mg-P/L) (Cieslik and Konieczka, 2017). Current P recovery techniques (e.g. as struvite) require concentrated streams containing at least 50 mg/L of P and research thus far has focused on developing strategies to concentrate P within wastewater treatment processes (Yuan et al., 2012).

All biological strategies developed to generate a concentrated stream of P from municipal wastewater, have thus far exploited the metabolic processes of polyphosphate accumulating organisms (PAOs). PAOs, are the driving force in enhanced biological phosphorus removal (EBPR) and have a unique metabolism. When exposed to aerobic or anoxic conditions, the PAOs uptake orthophosphate (PO_4^{3-}) from the surrounding environment and store it intracellularly as poly-P (Tarayre et al., 2016; Yuan et al., 2012). The P uptake takes place with a simultaneous oxidation of intracellular polyhydroxyalkanoates (PHA) (Lee et al., 2001). This results in a net removal of PO_4^{3-}-P from municipal wastewater. When PAOs with intracellular poly-P are exposed to a soluble organic carbon (C) source (e.g. acetate) under anaerobic conditions, carbon reserves are replenished, utilising energy derived from hydrolysis of the

stored poly-P (Kapagiannidis et al., 2013). As a consequence, PO_4^{3-}-P is released back into the environment (Chuang et al., 1996). Wong et al. (2013) strategically facilitated this second step of the EBPR process in a separate smaller volume of liquid and, based on this principle, they developed a method to achieve a concentrated P stream. With repeated use of this recovery stream in the EBPR process, the authors were able to achieve a P concentration of up to 100 mg-P/L. Wong et al. (2013) combined this P recovery strategy with post denitrification to maximise the use of carbon, not only to promote nitrate removal but also for the recovery of P from wastewater.

The need to add external carbon to facilitate post-denitrification presents a significant operational cost to the wastewater industry. Hence, the wastewater industry is constantly examining strategies to maximise the use of naturally abundant carbon in municipal wastewater to remove both nitrogen (N) and P from wastewater. Simultaneous nitrification-denitrification and P removal (SNDPR) is an elegant process that can achieve biological nutrient removal from wastewater at a lower carbon demand (Zeng et al., 2003). In SNDPR, N removal largely takes place via the nitrite pathway. It has been demonstrated that both denitrification and P removal take place with the aid of denitrifying PAOs (DPAOs, use nitrate or nitrite as final electron acceptors for P uptake) or denitrifying glycogen accumulating organisms (DGAOs, use nitrate or nitrite as final electron acceptors). The abundance of both the nitrite pathway (for N removal) and DPAOs (for both denitrification and P removal) in SNDPR, significantly reduces the demand for oxygen, decreasing aeration costs. This, in turn, helps conserve naturally occurring carbon, enabling its use to successfully remove both N and P from wastewater (Wang et al., 2015; Zeng et al., 2003).

There are many studies that have demonstrated the effectiveness of SNDPR to remove C, N, and P to very low levels (Table 1). However, none have demonstrated that SNDPR could facilitate P recovery, using the carbon naturally present in wastewater. Among lab-scale studies

carried out, there are only a handful of studies that have used a synthetic feed that resembled municipal wastewater in terms of C, N and P concentrations. Moreover, these studies only achieved low P concentrations at the end of the anaerobic phase (P release). Further, the incomplete removal of N and P also raise questions on whether P recovery from municipal wastewater containing low C concentrations could actually be achieved without the need for external C. For example, even with a higher concentration of C (chemical oxygen demand (COD) 400 mg/L) in the influent, Wang et al. (2016b) only achieved a P concentration of ~ 25 mg/L at the end of the anaerobic phase of their reactor cycle. Similarly, Jia et al. (2013b) only achieved a P concentration of 17 mg/L. Nonetheless, several non-SNDPR lab-scale studies have proven the feasibility of achieving higher P concentrations (~ 100 mg-P/L) using low COD (e.g. 200 mg/L) and P (10 mg/L) concentrations in the influent (Barat et al., 2008). However, since these studies ignored nitrogen removal (by using a nitrification inhibitor (allyl-N thiourea)), it still remains unclear whether low COD concentrations could facilitate simultaneous N removal and P recovery.

The aim of this study was to explore the use of a granular SNDPR process to recover influent P in a very small volume as a highly-concentrated P liquor (e.g. recovery of 10 mg-P that is in 1 L of influent in a volume of 100 ml (concentration - 100 mg-P/L)). There is a natural release and uptake of PO_4^{3-}-P during SNDPR operation and the study aimed to optimise the SNDPR process to maximise PO_4^{3-}-P release during the anaerobic phase of the sequencing batch reactor (SBR) cycle. As previously mentioned, SNDPR studies with C, P and N ratios of a typical wastewater influent have only managed to achieve PO_4^{3-}-P concentrations of 70 mg-P/L during anaerobic P release. Higher P concentrations have only been achieved with influent C concentrations greater than 400 mg-COD/L (Jia et al., 2013b; Zeng et al., 2003). Such high concentrations of C in the influent are not observed in typical municipal wastewater (Azizi et al., 2013). Hence, this study specifically examined the feasibility to use typical municipal

wastewater carbon concentrations to achieve higher release of PO_4^{3-}-P during the anaerobic phase of reactor operation.

A laboratory-scale SBR reactor was operated for a period of 4 months under alternating anaerobic / aerobic conditions. A synthetic medium, replicating concentrations of ammonia, P and carbon typical of municipal wastewater influent was used as an influent. Dissolved oxygen (DO) concentrations, volume exchange ratio, and biomass wasting were carefully managed to promote the growth of granular biomass. The performance of the reactor was closely monitored in terms of aerobic / anoxic P, N removal, anaerobic P release and microbial community changes.

2. Materials and methods

2.1 Sequencing batch reactor operation

A laboratory-scale SBR reactor with a working volume of 4 L was operated at room temperature (20 - 22˚C) under alternating anaerobic/ aerobic conditions (Fig. 1). The reactor was seeded with waste activated sludge (WAS, 2 L) collected from a local municipal wastewater treatment plant (Subiaco, WA, Australia). The inoculum had a mixed liquor suspended solids (MLSS) concentration of approximately 4.0 g/L. The operational cycle included a 2 h anaerobic period with 5 min of feeding (synthetic medium), 2 h of aerobic period, 20 min settling and 10 min decanting. Sodium salts of Acetate and propionate were alternately used (bi-weekly) as the carbon source to facilitate the enrichment of PAOs (Lu et al., 2006). At the beginning of the anaerobic phase, 2.8 litres of synthetic wastewater was pumped into the reactor, enabling a volume exchange ratio of 70 %, which was considered desirable for the enrichment of the granules. The hydraulic retention time (HRT) and solid retention time (SRT)

were maintained at 9 h and 20 days, respectively. The reactor was operated for a period of 110 days.

National Instrument hardware (CompactRIO) and software (Labview) were used to control, monitor and automate the operation of the reactor. Mixing was achieved at 50 rpm using an overhead stirrer (RZR2020, Heidolph, Germany). Maintenance of DO at set point was achieved using a luminescent DO probe (PDO_2; Barben Analyser Technology, USA) and the Labview software by switching on and off a solenoid valve connected to a compressed air outlet. The DO level was maintained between 0.30 and 0.8 mg/L. An intermediate junction pH sensor (Ionode IJ44, Ionode Pty Ltd, Australia) and an intermediate junction redox sensor (Ionode IJ64, Ionode Pty Ltd, Australia) were also fitted into the reactor and their outputs were recorded online. The pH in the reactor was not controlled.

2.2 Synthetic medium

The 2.8 L synthetic feed was composed of 40 mL of solution A, 200 mL of solution B, 200 mL of solution C and 2.36 L of deionised water. The composition of these three stock solutions were as follows: Stock solution A (per L): 25.63 g CH_3COONa or 17.15 g CH_3CH_2COONa; Stock solution B (per L): 0.9 g $MgSO_4$, 3.05 g NH_4Cl, 7.5 mg Peptone, 7.5 mg Yeast extract, 142.5 mg $CaCl_2.2H_2O$, 30 mg ethylenediaminetetraacetic acid (EDTA), 4.5 mg $FeCl_3.6H_2O$, 0.36 mg $ZnSO_4.7H_2O$, 0.36 mg $MnCl_2.4H_2O$, 0.18 mg $Na_2MoO_4.2H_2O$, 0.09 mg $CuSO_4.5H_2O$, 0.45 mg $CoCl_2.6H_2O$, 0.54 mg KI, 0.45 mg H_3BO_3; Stock solution C (per L): 0.37 g KH_2PO_4, 0.65 g K_2HPO_4. The introduction of the feed at the beginning of the cycle, imposed a PO_4^{3-}-P concentration of 10 mg/L, an NH_4-N concentration of 40 mg/L and a COD concentration of 200 mg/L in the reactor.

2.3 Process monitoring and chemical analysis

Long-term performance monitoring of the reactor was carried out with routine influent and effluent sampling and mixed liquor suspended solids (MLSS) measurements. Influent and effluent samples were immediately filtered using 0.22 μm pore size syringe filters (Cat. No. SLGN033NK, Merck Pty Ltd, Australia) and stored at 4°C. The concentration of MLSS in the reactor was determined according to Standard Methods for the Examination of Water and Wastewater (Rice et al., 2012).

The reactor performance was monitored by conducting cyclic studies. The cyclic studies facilitated the monitoring of N removal and P uptake/release kinetics of the biomass. Each cyclic study involved withdrawing 2 ml of sample from the reactor every 15–30 min over the entire 6-h cycle. Each sample was immediately filtered using a 0.22 μm pore size syringe filter (Cat. No. SLGN033NK, Merck Pty Ltd, Australia). At the end of each cyclic study the MLSS of the reactor was determined in accordance to the Standard Methods for the Examination of Water and Wastewater (Rice et al., 2012). Additionally, 2 ml volumes of settled biomass was also collected (on 7, 23, 34, 62, 90 and 110 d of reactor operation) for microbial analysis.

The PO_4^{3-}-P, NH_4^+-N, NO_3^--N, NO_2^--N, Mg^{2+} and acetate concentrations in the filtered samples were determined using ion chromatography (ICS-3000, DIONEX). A Dionex ICS-3000 reagent free ion chromatography (RFIC) system equipped with an IonPac® AS18 4 x 250 mm column was used to measure acetate, nitrite and nitrate concentrations in liquid samples. Potassium hydroxide was used as an eluent at a flow rate of 1 mL/min. The potassium hydroxide concentration was 12-45 mM from 0-5 min, 45 mM from 5-8 min, 45-60 mM from 8-10 min and 60-12 mM from 10-13 min. Ammonium (NH_4^+-N) and Mg^{2+} were measured with the same RFIC but with a IonPac® CG16, CS16, 5 mm column. Methansulfonic acid was used as the eluent at a flow rate of 1 mL/min and a 30 mM concentration was maintained for 29 min.

The temperature of the two columns were maintained at 30°C. Suppressed conductivity was used as the detection signal (ASRS ULTRA II 4 mm, 150 mA, AutoSuppression® recycle mode).

2.4 Microbiological analysis

Biomass samples were taken from the reactor for microbiological analysis. Upon collection, the biomass samples were immediately stored in a -80 °C freezer. Subsequently, the samples were thawed at room temperature in preparation to extract DNA for 454 pyrosequencing. DNA extractions were carried out using the PowerSoil® DNA Isolation Kit (MO BIO Laboratories, Inc., USA) and stored at -20 °C until sequenced. DNA sequencing was carried out as previously described (Nagel et al., 2016).

In brief, the extracted DNA was quantified using a Qubit fluorometer, and 1-ng samples were amplified using the 16S ribosomal ribonucleic acid (rRNA) gene V4/5 primers (515F: GTGCCAGCMGCCGCGGTAA and 806R: GGACTACHVGGGTWTCTAAT) (Caporaso et al., 2010). Specifically, the above gene-specific primers were used with gene-specific primers tagged with Ion Torrent-specific sequencing adaptors and barcodes. The tagged and untagged primers were mixed at a ratio of 90:10. Amplification of all samples were restricted to 18–20 cycles minimising primer-dimer formation. Amplification was confirmed by agarose gel electrophoresis, and amplified products were quantified by fluorometry. Subsequently up to 100 amplicons were diluted to equal concentrations and adjusted to a final concentration of 60 pM. Templated Ion Sphere Particles (ISP) were then generated and loaded onto sequencing chips using an Ion Chef (Thermofisher Scientific) and sequenced on a PGM semiconductor sequencer (Thermofisher Scientific) for 650 cycles using a 400 bp sequencing kit that yields a

modal read length of 309 bp. Data collection and read trimming/filtering was performed using TorrentSuite 5.0.

2.5 Bioinformatics Pipeline

The Quantitative Insights into Microbial Ecology (QIIME) software package version 1.9.1 (Caporaso et al., 2010) was used for processing of the sequenced data. Three main files (454-machine generated FASTA file & quality score file and user generated mapping file) were used for downstream analysis in QIIME. The split_libraries.py script was used to separate reads in the FASTA file according to the mapping file. Chimeric sequence reads were thereafter identified and filtered using USEARCH61 and an unaligned reference SILVA database (Version 128) 97_otus_16S.fasta (Quast et al., 2013). Subsequently, operational taxonomic units (OTUs) were assigned at 97 % sequence similarity using the same reference database file from SILVA. Once a representative sequence was appointed for each OTU picked, a taxonomic assignment was carried out using the RDP classifier version 2.2 (Wang et al., 2007) in reference to MiDAS taxonomic database version 2.1 (McIlroy et al., 2015). The bacteria directory at MiDAS taxonomic database was further used to determine various metabolic groups.

3. **Results and discussion**

3.1 The reactor successfully established a stable biomass with good P uptake/release and N removal

Upon inoculation with activated sludge, approximately 3 months was required to develop a granular biomass that satisfactorily maintained low N, P and C (0 mg NH_4-N/L, 0.8 mg NOx-N/L, 0 mg PO_4^{3-}-P/L and 0 mg acetate/L) concentrations in the effluent. During the first 62

days, there was an attempt to maintain a DO level ranging from 0.4 to 0.6 mg/L in the aerobic period. However, the system failed to maintain the desired DO set point as over 2/3 of the cycle, the DO fluctuated between 0 and 0.4 mg/L (Fig. 3a). Nonetheless, this mode of operation still enabled a rapid improvement in the aerobic P uptake and anaerobic P release activity of the biomass. Specifically, both aerobic P uptake and anaerobic P release activities increased almost identically (approximately 2 to 20 mg PO_4^{-3}-P/g-MLSS) during the period of 0 – 62 days (Fig. 2a). This also coincided with an increase in both nitrification and denitrification activities. However, compared to the increase of aerobic P uptake and anaerobic P release activities, the increase of nitrification and denitrification activities were approximately 6 times lower (Fig. 2a). The MLSS only marginally fluctuated (4.18 ± 0.62 g/L) throughout the entire period of reactor operation (Fig. 2a) suggesting only a minor change in biomass concentration in the reactor. Since the amount of suspended solids in the synthetic feed was negligible, the values of MLSS were considered as close estimates of the biomass concentration in the reactor. The steady MLSS observed throughout reactor operation is a result of the high-volume exchange ratio (70 %) and SRT (20 d) maintained.

The results obtained thus far suggested that the increased nutrient removal was likely a result of an increase in either the abundance or the enzymatic activities of specific microbial communities. In fact, along with the increase of biological activity, the specific release and uptake rates of P also increased in the reactor (Fig. 2b). However, an increasing difference was noted between specific P release and uptake rates (Fig. 2b). The low specific P uptake rate, necessitated a prolonged exposure of biomass to aerobic conditions to enable a complete removal of P. Similarly, during the first 62 days, although nitrification and denitrification activities increased, specific nitrification and denitrification rates declined or remained analogous to day 7 (Fig. 2c). The low specific P uptake and the nitrification rates were most likely a result of the low DO concentration that prevailed during the aerobic period of the

reactor. Hence, from day 62 onwards, the DO set point of the reactor was increased to maintain a DO concentration of 0.3 – 0.6 mg/L throughout the aerobic period. This resulted in a gradual increase of specific P uptake rate (Fig. 2b). The increase of DO also enriched the PAOs (i.e. both aerobic and DPAOs) and by day 110, the overall PAO abundance reached 12 % (Fig. 2d). The increase of PAOs and DO facilitated similar specific P release / uptake rates (i.e. 24.14 and 23.52 mg-P/g.MLSS.h respectively) and this enabled a rapid removal of P during the aerobic/anoxic period of the reactor cycle.

The increase of DO also increased the specific nitrification and denitrification rates, which were 2.41 and 2.46 mg-N/g.MLSS.h respectively by day 110 (Fig. 2c). A marginal increase of the ammonia oxidising bacteria (AOB) and the nitrite oxidising bacteria (NOB) population was also observed once the DO concentration was increased in the reactor (Fig. 2e). The increase of denitrification rate correlated with an increase of DPAO and DGAO abundance in the reactor, suggesting that DPAOs and/or DGAOs were responsible for the observed increase of denitrification. An overall decrease in abundance of order Burkholderiales (a bacterial order known to contain denitrifiers (Thomsen et al., 2007)) from 22.5 to 7.25 % between days 34 and 110 (Fig. 2f) suggest a possible decrease in abundance of heterotrophic denitrifiers. The marginal increase of abundance and/or community shift of the order Rhodocyclales (a bacterial order known to contain DPAOs and DGAOs (Zhang et al., 2018)), on the other hand coincided with an increase of DPAOs and/or DGAOs in the reactor. Overall, the increase of nitrification, denitrification and P removal resulted in a low nutrient content in the reactor effluent.

3.2 The change of microbial composition influenced the reactor performance

Fig. 2f illustrates a large microbial community shift in the reactor between 7 and 34 days of operation. There was a gradual reduction in the relative abundance of the members of order

Rhodocyclales, which consists of microorganisms such as *Candidatus Accumulibacter* (a well-known PAO), *Propionivibrio* (a well-known GAO), *Thauera*, *Dechloromonas* and *Sulfuritalea* (which are known denitrifiers/aerobic heterotrophs) (Coyotzi Alcaraz, 2014; Lu et al., 2006; McIlroy et al., 21 - 26 Aug 2016). The relative abundance of glycogen accumulating organisms (GAOs) and other heterotrophs decreased and the relative abundance and / or activity of PAOs increased during this period (Fig. 2d). The result also corroborated with the rapid increase of P uptake/release activity recorded during this period (Fig. 2a).

On the other hand, the decrease in the relative abundance of the members of order Rhodocyclales coincided with an increase in abundance of the members of order Burkholderiales (Fig. 2f). Bacterial genera such as *Rhodoferax* and *Acidovorax* of Burkholderiales are known for their ability to denitrify (Thomsen et al., 2007). Hence, the increase in abundance of the members of order Burkholderiales may suggest an overall increase of heterotrophic denitrifiers and there was also a gradual increase of denitrification observed between 7 and 34 days of reactor operation (Fig. 2c). From 62 to 110 days of operation, a marginal community shift was observed at an order level and the overall bacterial community in the reactor remained stable.

3.3 The PAOs had a higher affinity towards oxygen

Two cyclic studies carried out on days 62 and 110 were compared to understand how an increase of DO would impact the overall performance of the reactor. The cyclic study carried out on day 62 (Fig. 3a) revealed a poor removal of NH_4^+-N. In this cyclic study, a clear bending point was recorded in the NH_4^+-N profile during the aerobic phase of the reactor cycle (Fig. 3a). During the initial 2 h of the aerobic cycle, the NH_4^+-N removal rate was 0.7 mg/g-MLSS.h. After 2 hours into the aerobic cycle, P was completely up taken by the PAOs and thereafter a

higher NH_4^+-N removal rate of 1.35 mg/g-MLSS.h was observed (Fig. 3a). Although the reactor was aerated during the entire aerobic phase, there was no measurable concentration of DO during the first 30 min of reactor operation (Fig. 3a). During this period, the removal of NH_4^+-N was insignificant when compared with the removal of PO_4^{3-}-P (Fig. 3a). This suggests that biological ammonia oxidation (driven by AOB) was compromised during this period, and PAOs appeared to have preferentially utilised all of the supplied oxygen to uptake P. An increase of DO in the reactor was only noted once PO_4^{3-}-P concentration decreased to approximately below 40 mg/L and this coincided with an increased AOB activity as reflected by the increased removal of NH_4^+-N.

The above observation implies that the acclimatised PAOs in the biomass had a higher affinity towards oxygen when compared with AOBs. Blackburne et al. (2008) however, showed that AOBs have a higher affinity towards oxygen in a study that was conducted to examine whether NOBs could be washed out in a continuous-flow reactor using DO concentration as the only selection factor. Similarly, Carvalheira et al. (2014) showed that PAOs also have a higher affinity towards oxygen in a study they carried out to examine the impact of aeration on PAOs and GAOs. Although both AOBs and PAOs are known to have higher affinities towards oxygen, to our knowledge no study has examined which of these two has the highest affinity towards oxygen in a single study. The findings of this study complement the findings of Yang et al. (2016) and provides indirect evidence that PAOs have a higher affinity towards oxygen compared to AOBs. Based on independent studies of Carvalheira et al. (2014) and Rongsayamanont et al. (2010), Yang et al. (2016) suggests that PAOs have a higher affinity towards oxygen when compared with AOBs. However, to be conclusive and comparative, the oxygen Monod half saturation constants (K_o), for both AOBs and PAOs should be determined against a single SNDPR sludge.

3.4 Dissolved oxygen (DO) concentration of 0.5 mg/L was essential to achieve complete nutrient removal and a high P concentration at the end of the anaerobic phase

Increasing the DO concentration in the reactor increased the availability of oxygen for both P uptake and nitrification to simultaneously occur from the start of the aerobic phase of the reactor cycle (Fig. 3b). An unchanged rate of NH_4^+-N reduction before and after completion of P uptake, suggests that nitrification occurred at its maximum rate during the entire aerobic period, implying that AOBs were not limited by oxygen. While the bulk of the NOx-N produced from nitrification was simultaneously removed, a small concentration was observed accumulating in the reactor (Fig. 3b). This small increase of NOx-N confirms that the denitrification rate was marginally lower than the nitrification rate in this reactor. Since the nitrification process was completed approximately 30 min prior to the end of the cycle, the remaining 30 min of the cycle was sufficient to completely remove NOx-N from the final effluent.

Maintaining a suitable oxygen gradient within the granule is essential for the described SNDPR process to occur. In this study, a DO concentration of approximately 0.5 mg/L (during the aerobic phase) was found to be suitable to achieve a similar nitrification and a denitrification rate. The bulk water DO concentration enabled both oxygen-demanding (i.e. P uptake by PAOs and nitrification by AOB) and denitrification reactions to simultaneously take place, enabling an efficient SNDPR process. The results further revealed that an increase in DO concentration to approximately 0.5 mg/L (due to a completion of P uptake), was not detrimental for a complete removal of N. This finding is noteworthy because DO concentrations in excess of 0.5 mg/L have been demonstrated as detrimental to the SNDPR process (Meyer et al., 2005). Specifically, elevated levels of DO were thought to further oxidise nitrite (NO_2^--N) into nitrate (NO_3^--N), increasing carbon requirements to remove P and NO_x^--N from the final effluent (Meyer et al., 2005; Zeng et al., 2004).

3.5 A low COD:N ratio similar to that of municipal wastewater can facilitate a high concentration of P release, enabling P recovery

The influent used in this study had C and N contents similar to that of a typical municipal wastewater, as characterised by a low COD:N ratio (here approximately five). To determine whether the SNDPR process had become more efficient at releasing P during the anaerobic phase, the specific P release rates were calculated (Fig. 2b). Clearly, the rate of P release during the anaerobic phase of the process increased gradually over the entire period of the study, reaching a maximal rate of 24 mg/g.MLSS.h at day 110 (Fig. 2b). Further, during the initial days of reactor operation, the COD: $P_{released}$ ratio was high, approximately 25 (at day 7). However, a more than 10-fold decrease of this ratio was achieved after 110 days of operation (to 2.35), signifying that the biomass became more efficient in using the influent carbon to facilitate P release (Fig. 3a & b). Upon achieving this low COD: $P_{released}$ ratio, the PO_4^{3-}-P concentration increased to 100 mg/L at the end of the anaerobic phase of the cycle (Fig. 3a & b). Given that the influent PO_4^{3-}-P concentration was low (10 mg/L), the ability of the described process to increase the PO_4^{3-}-P concentration by 10-fold (i.e. reaching ~100 mg P/L at end of the anaerobic phase) creates an opportunity to recover influent PO_4^{3-}-P in a smaller volume as a concentrated PO_4^{3-}-P liquor.

To our knowledge, this is the first study to demonstrate that a high concentration of PO_4^{3-}-P (up to 100 mg-P/L) can be achieved in a SNDPR process using a wastewater influent with a low COD/N ratio of five (COD concentration of 200 mg/L and an NH_4^+-N concentration of 40 mg/L) (Table 1). This is a notable finding, as earlier studies with similar low COD/N ratios have only demonstrated a low PO_4^{3-}-P release of approximately ~40 mg/L (Wang et al., 2015; Wang et al., 2016b) (Table 1). Given that municipal wastewater typically contains only low

concentrations of biodegradable COD, the results of this study highlight the potential of using SNDPR to promote P recovery while achieving excellent removal of nutrients and C from municipal wastewater. Nonetheless, further studies using real municipal wastewater as influent are required to validate the current findings.

3.6 Are DGAOs primarily responsible for denitrification?

In the SNDPR process, organic carbon is introduced into the reactor during the anaerobic phase of the cycle. In this study, the organic carbon (acetate or propionate) in the synthetic wastewater was fully consumed and stored by the granular biomass (Fig. 3) in the complete absence of any electron acceptor. Hence, PAOs, DPAOs, GAOs and/or DGAOs are the likely organisms that stored majority of the COD that prevailed in the influent.

The observed denitrification in the described process took place both during and after P uptake was completed (Fig 3). In the absence of P and a source of carbon, denitrification could only take place with the assistance of DGAOs, whereas denitrification during P uptake may have been a result of both DPAOs and DGAOs. Fig 4 shows a gradual increase in the denitrification rates before and after P exhaustion (i.e. in the presence and absence of P) during the acclimatisation period in the reactor. During the early operation of the reactor (i.e. 62 d), the denitrification rates observed in the presence of P were an order of magnitude higher compared to the denitrification rates observed in the absence of P (Fig. 4). According to the cyclic study on day 62 (Fig. 3a), the accumulation of NOx-N specifically after exhaustion of P, was a result of the low denitrification rate and/or a higher nitrification rate. The nitrification rate, however, only increased marginally (0.2 mg/L.h) after exhaustion of P (Fig. 3a) and hence, the accumulation of NOx-N resulted from the reduction of the denitrification rate. This suggests that in addition to DGAOs, DPAOs were also likely to be contributing towards denitrification

when P was present in the reactor. The reduced denitrification rate observed in the absence of P (i.e. after all the PO_4^{3-}-P was up taken by PAOs or DPAOs) was most likely due to the inability of DPAOs to denitrify and this also suggests DPAOs reliance on P to remain active in the reactor (Fig. 4). During the final days of reactor operation, the denitrification rates observed in the absence of P far exceeded denitrification rates observed in the presence of P (Fig. 4). This is a result of an increased DGAO activity and/or an increased DGAO abundance. A microbial analysis confirmed an increase of DGAO abundance from approximately 1.2 to 4.5 % between 62 and 110 days of reactor operation. Although chemical data does not suggest an increase or a decrease of DPAO activity, the microbial analysis indicated a 1.5 % reduction in the abundance of DPAOs during the final 20 days of reactor operation. This 1.5 % decline in DPAO abundance coincided with a 1.3 % increase of DGAO abundance and this overall facilitated a bacterial community that enabled complete removal of N, P and C from the influent wastewater.

4. **Conclusions**

This study examined whether a SNDPR process could facilitate recovery of P as a concentrated liquor at the end of the anaerobic phase of the reactor cycle. Based on the results the following can be concluded.

- A PO_4^{3-}-P concentration as high as 100 mg/L is achievable at the end of the anaerobic phase of the reactor cycle. This concentrated stream of PO_4^{3-}-P enabled recovery of influent PO_4^{3-}-P (10 mg/L) in a very small volume as a concentrated liquor and enables economies of scale to recover P specifically as struvite (Adnan et al., 2003).
- A COD concentration of 200 mg/L is adequate to create a concentrated stream of PO_4^{3-}-P suitable for P recovery at the end of the anaerobic phase of the reactor cycle. The

use of COD to create such a high concentrated stream of P did not hinder effective removal of NOx-N. Specifically, C was not found to be a limiting factor for denitrification. This is the first study to demonstrate opportunities to recover P with a COD concentration that is typical of municipal wastewater.

- Maintenance of a DO concentration of below 0.5 mg/L was critical to achieve a balanced microbial community in the granules of the SNDPR process.
- PAOs appear to have a higher affinity towards oxygen than nitrifiers, and an early completion of P uptake and a similar rate of nitrification and denitrification appear to be important to achieve good nutrient removal.
- DGAOs played a major role in the SNDPR process, specifically to remove NOx-N.

Acknowledgments

This project was funded by CSIRO Land and Water. Curtin University is acknowledged for the Curtin University Postgraduate Student Association (CUPSA) academic grant provided to Sara Salehi Lashkajani. The authors also would like to thank Dr Anna Kaksonen, Dr Haylea Miller and Ana Mesquita from CSIRO Land and Water for their valuable comments to the manuscript.

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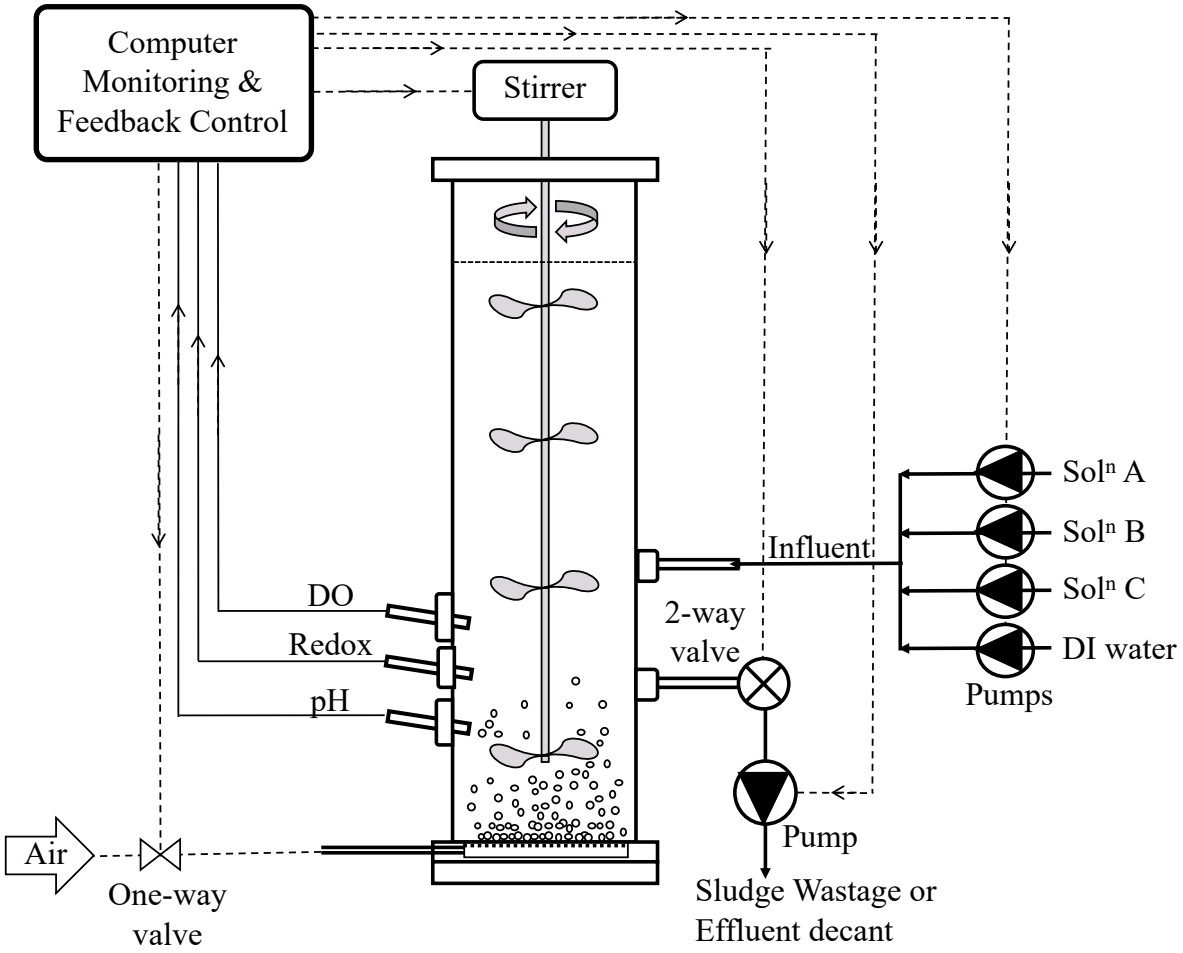


Fig. 1. Schematic of SBR reactor

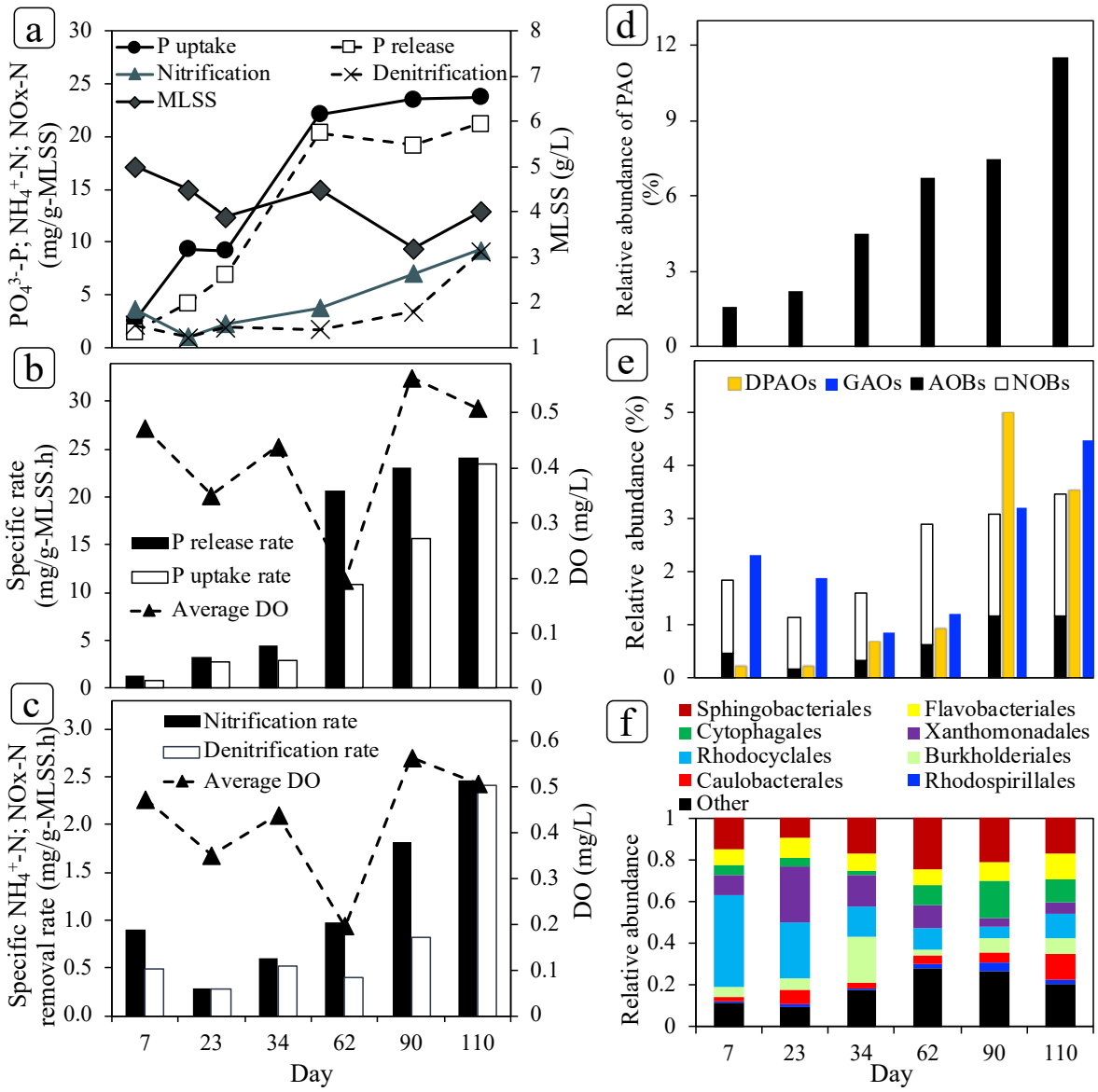
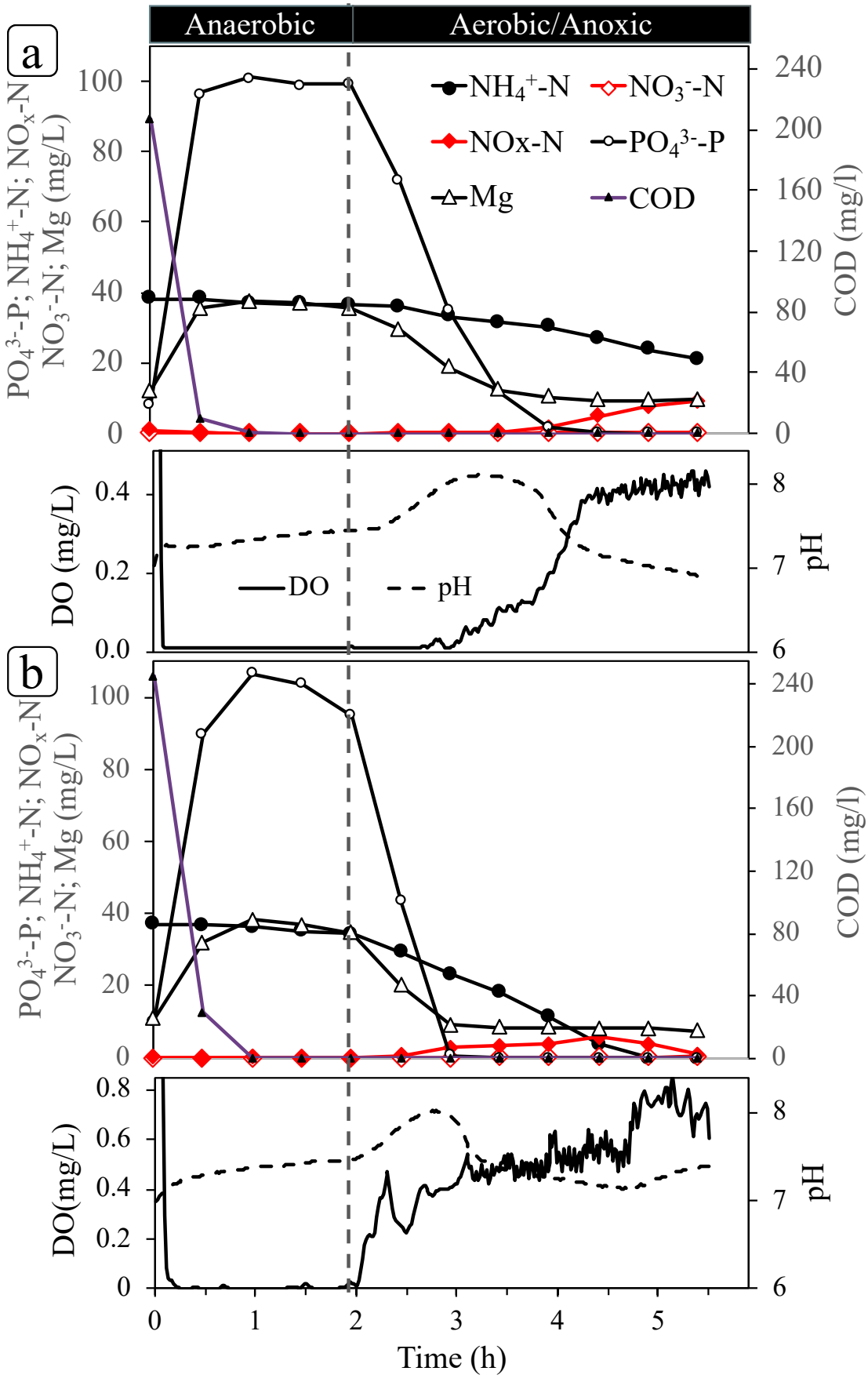


Fig. 2. (a) P uptake/release and nitrification/denitrification activity changes in the reactor; (b) P uptake and release rates in the reactor; (c) Nitrification and denitrification rates in the reactor, (d) The abundance and shift of bacteria classified to the order level; (e) Relative abundance of PAOs; (f) Relative abundance of DPAOs, GAOs, AOBs, NOBs.



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573 **Fig. 3.** Cyclic studies carried out on (a) 62 d and (b) 110 d

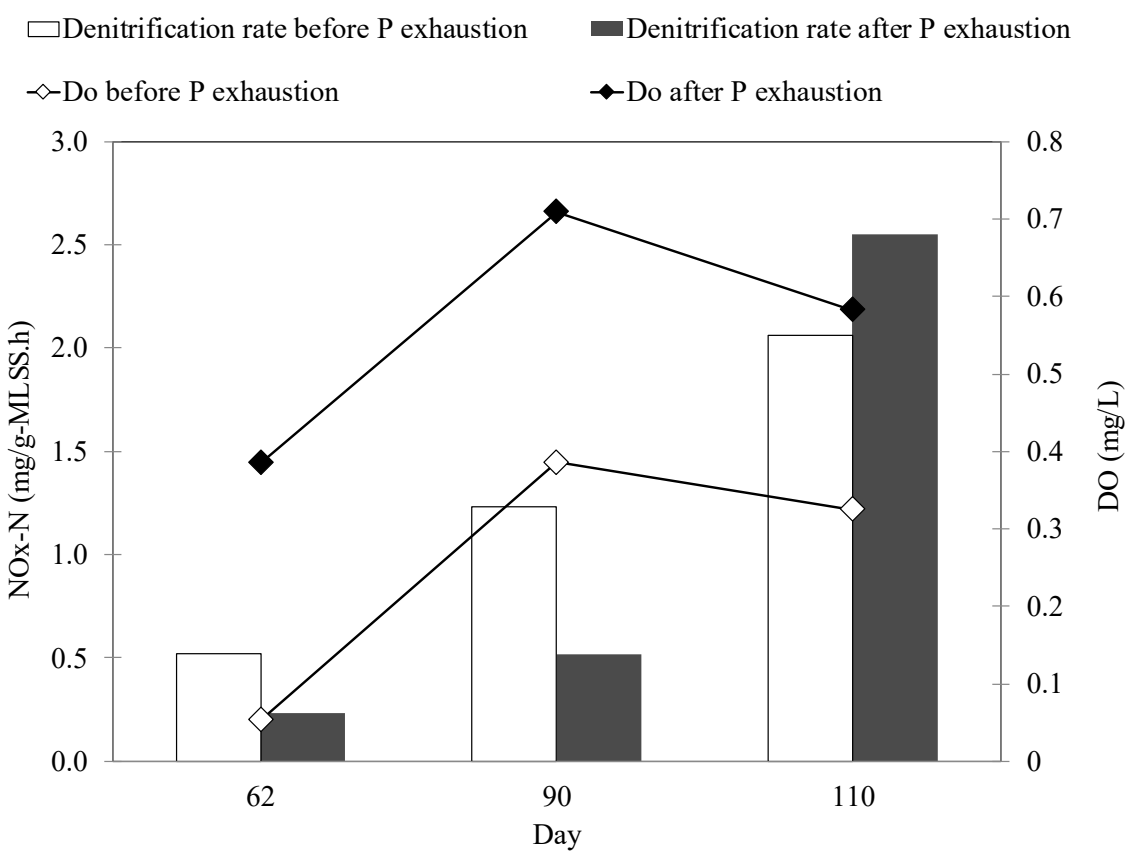

Fig. 4. Denitrification rates in the presence and absence of P

Table 1. A comparison of SNDPR studies

	Operational Parameters													Wastewater Characteristics					Performance			
Research	Working vol (L)	Cycle length (h)	Anaerobic phase (h)	Aerobic phase (h)	Settling time (min)	Decant vol (L)	MLSS (g/L)	DO (mg/L)	Temperature (° C)	Carbon source	HRT (h)	SRT (day)	pH	COD (mg/L)	NH_4^+-N (mg/L)	PO_4^{3-}-P (mg/L)	COD/N	COD/P	Max P release (mg/L)	SND efficiency (%)	P removal (%)	NOx-N in the effluent (mg/L)
(Lu et al., 2016)	8	6.0	2.2	2.7	60	2.0	2.2-3.5	0.8-1.6	20	NaAc	24	8	7.5	800	50	36	16	22	75	57	100	3
(Zeng et al., 2003)	4	4.8	1.0	3.0	43	2.0	3.3	0.5-0.6	18-22	NaAc	9.6	15	7-7.5	400	40	15	10	27	75	98	100	0
(Lemaire et al., 2006)	5	6.0	1.5	3.6	40	3.0	3.9-4.6	0.4-0.5	20-22	NaAc	10	20	7-7.5	230	23	10	10	23	110		100	0
(Jia et al., 2013a)	5	6.0	1.5	3.0	70	3.0	3.0-3.5	0.4-0.8	25	Glucose/NaAc	10	20		400	40	4.2	10	95	12	81	86	7.6
																10		40	17	80	87	7.9
																14		28	30	86	89	5.7
(Wang et al., 2016a)	8	6.0	1.5	3.5	45	4.0	3.2-3.5	0.4-0.7	25	NaAc	12	16	7-7.5	400	40	15	10	26.7	23	53	90	9.2
									10										25	84	91	5.4
(Li et al., 2008)	4	8.0	2.0	3.0	60	1.8	3.5	0.2-0.5	21	NaAc	16	22	7.3-8	300	35	12	9	25	68	53	81	8
										Propionate/NaAc: 1/1									60	63	94	6.5
										Propionate/NaAc: 2/1									58	79	97	3.5
(Meyer et al., 2005)	5	6.0	1.95	3.1	43	3.0		0.4-0.5		NaAc	10	15	7-7.5	136	18	11	8	13			100	3.5
(Jia et al., 2013b)	15	6.0	1.5	3.0	70	7.5	3.0-3.3	0.4-0.8	25	Glucose/NaAc	12	20	7-7.5	350	50	5	7	70	12	92	82	4
(Wang et al., 2015)	8	6.0	3.0	2.5	20	3.0	3.0	1.0		NaAc	14.6	10.9	7.2-8	254	65	6	4	42	26	49	94	10
This Study	4	6.0	2.0	4.0	20	2.8	3.0	0.5	22	NaAc			7-8	200	40	10	5	20	100	100	100	1.2