



Combining simultaneous nitrification-endogenous denitrification and phosphorus removal with post-denitrification for low carbon/nitrogen wastewater treatment



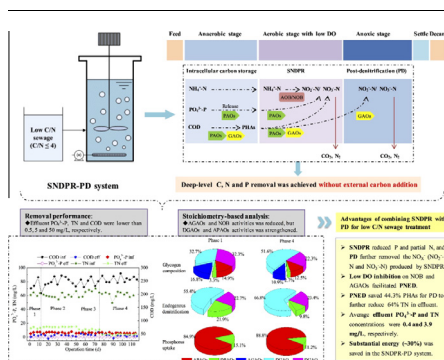
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HIGHLIGHTS

- The first combination of SNDPR with PD for low C/N ratio (<4) wastewater treatment.
- Low DO inhibition on NOB and AGAOs facilitated PNED.
- Highly enriched PAOs, GAOs, AOB over NOB facilitated PNED, P uptake, and PD.
- PNED saved 44.3% PHAs for PD to further reduce 64% TN in effluent.
- Average effluent PO_4^{3-} -P and TN concentrations were 0.4 and 3.9 mg/L, respectively.

GRAPHICAL ABSTRACT



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ABSTRACT

Due to the limited nutrient removal from low carbon/nitrogen (≤ 4) wastewater, a process combined simultaneous nitrification-endogenous denitrification and phosphorus removal (SNDPR) with post-denitrification (PD) in a SBR was proposed for deep-level nutrient removal without external carbon addition. SNDPR driven by PAOs and GAOs reduced PO_4^{3-} -P (98.3%) and partial TN (59.0%) at low DO conditions (0.5 ± 0.1 mg/L), and post-denitrification achieved further NO_3^- (produced by SNDPR) removal (24.0%) anoxically by utilizing the residual intracellular polymers in GAOs. Combined control of anaerobic/aerobic/anoxic durations and low DO inhibition to aerobic GAOs and NOB conducted partial nitrification-endogenous denitrification (PNED) (66%), which saved 44.3% intracellular polymers to further reduce 64% TN in effluent. After 115-day operation, the average effluent PO_4^{3-} -P and TN concentrations were 0.4 and 3.9 mg/L, respectively, with 92.1% of TN removal. Highly enriched PAOs ($36\% \pm 2\%$), GAOs ($22\% \pm 2\%$) and AOB ($15\% \pm 3\%$) over NOB ($3\% \pm 1\%$) facilitated P uptake, PNED and post-denitrification in the SNDPR-PD system.

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1. Introduction

In wastewater treatment plants (WWTPs), phosphorus (P) removal always accompanies with nitrogen (N) removal, both

requiring organic carbon which is often limiting (Zhang et al., 2013). Simultaneous nitrification-endogenous denitrification and phosphorus removal (SNDPR) was reported to efficiently utilize the carbon source in raw wastewater by strengthening the anaerobic intracellular carbon storage (Wang et al., 2015). At the anaerobic stage, phosphorus- and glycogen accumulating organisms (PAOs and GAOs) take up carbon sources in influent, primarily volatile fatty acids (VFAs), and store them in the form of poly-

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hydroxyalkanoates (PHAs), with the energy gained from the degradation of glycogen (Gly) with/without the degradation of polyphosphate (Oehmen et al., 2007; Pijuan et al., 2010). At aerobic stage, PAOs and GAOs regenerate Gly with/without P uptake using the stored PHAs, and ammonia and nitrite oxidizing bacteria (AOB and NOB) conduct nitrification. More importantly, both denitrifying PAOs-and-GAOs (DPAOs and DGAOs) conduct N removal at the aerobic stage (Zeng et al., 2003a; Meyer et al., 2005; Wang et al., 2015). Thus, simultaneous N and P removal is achieved in SNDPR systems.

However, at the aerobic stage of SNDPR system, PAOs, GAOs, AOB and NOB compete for dissolved oxygen (DO), which always result in incomplete ammonia oxidation and excess intracellular carbons waste without contributing to endogenous denitrification (Wang et al., 2015). Both nitrite and nitrate were the nitrification products by AOB, which would later become the electron acceptors for DGAOs, DPAOs and NOB. Compared with nitrite pathway, endogenous denitrification via nitrate consumes 50% more PHAs (Wang et al., 2016). Suitable DO concentration and aerobic duration are particularly the case for SNDPR optimization and nutrient removal via nitrite. Previous reports on SNDPR optimizations were just accomplished by adding aerobic granular (Bassin et al., 2012; Coma et al., 2012) or biofilms (Wang et al., 2009a; Yang et al., 2010) or extending anaerobic durations to enhance SND (de Kreuk et al., 2005; Wang et al., 2015), and the $\text{PO}_4^{3-}\text{-P}$ and TN removal efficiencies were still low (52% and 71% in Wang et al. (2009); 82% and 84% in Yang et al. (2010); 80% and 30% in Bassin et al. (2012); 75% and 93% in Coma et al. (2012); 78% and 94% in Wang et al. (2015)). Therefore, highly efficient sewage treatment process based on SNDPR and control strategies should be developed for advanced nutrient removal.

Post-denitrification (PD) process driven by GAOs could achieve N removal anoxically using both PHAs and Gly (Qin et al., 2005; Coats et al., 2011). Considering that SNDPRs always contains high amounts of PHAs and Gly due to the highly enriched GAOs and PAOs (Wang et al., 2015; Coma et al., 2012), it is extremely significant to achieve advanced N and P removal via full utilization of intracellular polymers stored in PAOs and GAOs by combining SNDPR with post-denitrification (termed as SNDPR-PD). On the one hand, the SNDPR could reduce $\text{PO}_4^{3-}\text{-P}$ and partial TN at low aeration intensity; on the other hand, the post-denitrification could further remove the NO_x^- (NO_2^- -N and NO_3^- -N) produced by SNDPR, and thus reducing the carbon demand for denitrifying OHOs and leading to more carbon sources available for SNDPR-PD. For now, post-denitrification driven by PHAs and Gly has been reported for nitrogen removal from landfill leachate treatment (Li et al., 2014; Miao et al., 2015). Landfill leachate contains high amounts of organic substances and low amounts of phosphorus, making it suitable to enrich GAOs to conduct endogenous denitrification (Miao et al., 2015). Post-denitrification process with/without SNDPR for the treatment of real domestic wastewater with low carbon/nitrogen ratio (C/N, referred to chemical oxygen demand (COD)/total nitrogen (TN)) (≤ 4) without external carbon addition has not been reported.

This study aims at developing a SNDPR combined with post-denitrification process in a single sequencing batch reactor (SBR) and exploring the feasibility of the system for deep-level C, N and P removal from low C/N ratio (≤ 4) wastewater without external carbon addition. By comprehensively regulating the anaerobic, aerobic and anoxic durations and DO concentration, the SNDPR was firstly optimized and lately combined with post-denitrification, during which the P uptake, PHAs degradation, Gly production, SND efficiency and effluent TN concentrations were monitored and stoichiometry-based analyzed to evaluate the enhanced nutrient removal performance. The enhanced nutrient removal was also verified by the population dynamics and activity

variations of functional microorganisms. Finally, performance of the SNDPR-PD system was compared with other related systems to demonstrate its superiority.

2. Materials and methods

2.1. SBR operation for the SNDPR-PD system

A laboratory-scale open-mouthed SBR with a working volume of 8 L and fed with domestic wastewater was used in this study (Fig. 1). The SBR was operated at room temperature, and operated under extended anaerobic, short aerobic conditions with low DO concentration and anoxic conditions. During all anaerobic, aerobic and anoxic periods, an agitator was used to keep the sludge in suspension. During the aeration period, DO concentration was stably maintained using an online real-time control device (PLC). In each cycle, 3 L domestic wastewater was added into the reactor in the beginning of the anaerobic stage; 200 mL of mixed liquor was removed in the end of the aerobic stage to achieve a mixed liquor volatile suspended solid (MLVSS) level of 2200 ± 500 mg/L. More detailed operation conditions of the SBR were shown in Table 1.

2.2. Domestic wastewater and seeding sludge

Domestic wastewater was taken from a septic tank in the residential area of Beijing University of Technology (Beijing, China). The wastewater has a low C/N ratio (average 3.5) with COD 203.8–281.6 mg/L, VFAs 123.2–182.8 mg/L (acetic acid 118.1–174.4 mg/L, propionic acid 1.8–6.2 mg/L, n-butyric <5 mg/L, iso-butyric acid <5 mg/L, iso-valeric <3.5 mg/L), BOD_5 114.8–177.3 mg/L, $\text{NH}_4^+\text{-N}$ 48.4–69.0 mg/L, NO_2^- -N <1 mg/L, NO_3^- -N <1 mg/L, $\text{PO}_4^{3-}\text{-P}$ 5.1–7.9 mg/L, and TN 61.4–79.5 mg/L. The activated sludge inoculated was taken from an ongoing lab-scale SBR system (working volume: 8L) which had achieved a stable performance of biological N and P removal for 6 months (Wang et al., 2015).

2.3. Methods for chemical analysis

Mixed liquor samples were filtered through 0.45 μm filter paper before analysis. $\text{NH}_4^+\text{-N}$, NO_2^- -N, NO_3^- -N, and $\text{PO}_4^{3-}\text{-P}$ were analyzed using Lachat Quik-Chem8500 Flow Injection Analyzer (Lachat Instrument, Milwaukee, USA). BOD_5 , COD, MLSS, MLVSS, sludge volume index (SVI) and SV% were analyzed according to standard methods (APHA, 1998). VFAs were analyzed using a gas chromatograph (GC, Agilent 7890). TN was analyzed using a TN analyzer (Multi N/C3000, Ananlitijena AG, Germany). PHAs were determined by the sum of PHB and PHV, both PHB and PHV were analyzed according to Oehmen et al. (2005). Glycogen was analyzed according to Zeng et al. (2003b). Scanning electron microscopy (SEM) was conducted to elucidate the microscopic behavior of activated sludge.

2.4. Methods for microbial community identification

Fluorescence *in situ* hybridization (FISH) was used to quantify AOB, NOB, PAOs and GAOs (Amann et al., 1990), and quantification of DPAOs in PAOs was calculated by Wachtmeister et al. (1997). FISH probes used were: EUB_{mix} (comprising equal amounts of EUB338, EUB338-II, and EUB338-III) for most *Eubacteria*; PAO_{mix} (comprising equal amounts of PAO462, PAO651, and PAO846) for *Accumulibacter*; GAO_{mix} (comprising equal amounts of GAO431 and GAO989) for *Competibacter* (Wang et al., 2015); NSO190 for β -proteobacterial AOB specific, NSO1225 for *Nitrosomonas* spp.,

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