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# Combining partial nitrification and post endogenous denitrification in an EBPR system for deep-level nutrient removal from low carbon/ nitrogen (C/N) domestic wastewater



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G R A P H I C A L A B S T R A C T

#### HIGHLIGHTS

- The combination of partial nitrification, PED and EBPR in an AOA operated SRR was achieved
- Partial nitrification saved carbons consumption for PED to achieve 94.9% TN removal.
- EBPR ensured 92.4% PO<sub>4</sub><sup>3</sup>-P removal.
- Effluent PO<sub>4</sub>-P and TN of 0.4 and 3.0 mg/L were realized at C/N of 3.1.
- Subsequent utilization of Gly (after PHAs) in DGAOs facilitated deeplevel TN removal.

ABSTRACT

In this study, partial nitrification and post endogenous denitrification (PED) were combined with enhancing bacterial phosphorus removal (EBPR) in an anaerobic/aerobic/anoxic operated sequencing batch reactor (SBR) for deep-level nutrient removal from low carbon/nitrogen (C/N, chemical oxygen demand (COD)/total nitrogen (TN)) domestic wastewater. At anaerobic stage, abundant organic matters (96.6% of COD consumption) in raw wastewater were stored as poly-hydroxyalkanoates (PHAs) by phosphorus and glycogen accumulating organisms with enhanced activities, which provided sufficient intracellular carbons for subsequent aerobic phosphorus uptake and anoxic PED. By controlling suitable aeration rate and duration, high nitrite accumulation rate (97.2%) was obtained at aerobic stage, which saved intracellular carbons consumption of PED. Moreover, the subsequent utilization of glycogen after PHAs via PED ensured the deep-level TN removal (94.9%) without external carbon addition. After 160day operation, the average effluent  $PO_4^3$ -P and TN concentrations were 0.4 and 3.0 mg/L, respectively, at C/N of 3.1.

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wastewater treatment plants for nitrogen (N) and phosphorus (P) removal. The conventional N removal process includes an aerobic

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#### 1. Introduction

Biological nutrient removal (BNR) has been widely applied in

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nitrification and an anoxic denitrification. Nitrification is a two-step autotrophic biological oxidation process driven by ammonia oxidizing bacteria (AOB, ammonia (NH $_4^+$ -N)  $\rightarrow$  nitrite (NO $_2^-$ -N)) and nitrite oxidizing bacteria (NOB,  $NO_2^-$ -N  $\rightarrow$  nitrate ( $NO_3^-$ -N)) sequentially, and denitrification is conducted by denitrifying ordinary heterotrophic organisms (DOHOs,  $NO_3^-N \to NO_2^-N \to dinitrogen gas (N_2)$ ) using exogenous organic carbons (Peng and Zhu, 2006). In nitrification process, both  $NO_2^-N$  and  $NO_3^-N$  are the oxidation products, but  $NO_2^-N$  is uncommonly accumulated due to its instant oxidation to  $NO_3^-N$  by NOB. Considering that these two substrates could be both removed via denitrification, partial nitrification-denitrification ( $NH_4^+-N \to NO_2^--N \to N_2$ ) provides an alternative for sustainable N removal compared with the  $NO_3^-N$  pathway (Peng and Zhu, 2006).

For now, partial nitrification-denitrification has gained much attention, since it could not only reduce the aeration requirement (by 25%) but also save the organic carbon consumption (by 40%) (Peng and Zhu, 2006). Recent research has also highlighted the strategies for achieving partial nitrification by inhibiting and washing out NOB based on its lower growth than AOB (Yang et al., 2007). Additionally, by using the real-time control based on indirect parameters (e.g. dissolved oxygen (DO) and pH), stable partial nitrification was always achieved (Guo et al., 2009; Yang et al., 2007). However, in partial nitrification-denitrification processes, pre-denitrification requires mixing the regurgitant NO<sub>2</sub>-N with raw wastewater, which always results in excess NO<sub>2</sub>-N discharged and certain carbon sources wasted (Zhang et al., 2013); while postdenitrification needs external carbon supplement (e.g. methanol and ethanol) to achieve the N removal, which obviously costs high (Guo et al., 2016b). Therefore, high-efficiency and low-cost sewage treatment process based on partial nitrification-denitrification still needs to be developed for advanced N removal.

The conventional P removal is accomplished by anaerobic P release and aerobic P uptake by phosphorus accumulating organisms (PAOs) in an enhancing biological phosphorus removal (EBPR) system. Under anaerobic condition, PAOs store organic matters as poly-hydroxyalkanoates (PHAs) with energy gained from both polyphosphate hydrolysis and glycogen (Gly) glycolysis. Under aerobic condition, PAOs conduct excessive orthophosphate ( $PO_4^{3}$ -P) uptake by degrading the stored PHAs and using oxygen as the electron acceptor (Smolders et al., 1995). Given this, competitions for organic carbon matters between PAOs and DOHOs might lead to unsatisfied nutrient removal under the carbon limitation (low C/N, referred to chemical oxygen demand (COD)/total nitrogen (TN) lower than 4) of wastewater (Wang et al., 2015; Yang et al., 2017). Additionally, another group of organisms known as glycogen accumulating organisms (GAOs), which exhibit similar metabolisms to PAOs but without P cycling, were also found in EBPR process and considered undesirable (Saunders et al., 2003).

Recently, the denitrifying GAOs (DGAOs) has been reported to achieve N removal by utilizing the intracellular endogenous carbon sources (PHAs or/and Gly) stored at anaerobic condition (Qin et al., 2005; Zeng et al., 2004). Compared with exogenous carbon sources, the endogenous ones are more advantageous in the postdenitrification process, due to the obvious reduction in operation costs and efficient realization in deep-level N removal. Thus, it would be extremely significant to combine partial nitrification with post-endogenous denitrification (PED) in an EBPR system for advanced nutrient removal from low C/N wastewater without external carbon addition. On the one hand, partial nitrification could reduce the organic carbons demand for PED, leading to certain amounts of influent COD available for EBPR to achieve P removal. On the other hand, endogenous denitrification at the post anoxic stage could reduce the NO2-N in effluent, and thus reducing the COD consumed by DOHOs and leading to more carbon sources available for EBPR and PED. Moreover, PAOs and DGAOs in the combined system could fully utilize the influent COD for synthesizing intracellular carbons storage, which makes nearly none COD being oxidized or wasted under aerobic conditions.

For now, post-endogenous denitrification driven by DGAOs has been investigated in biofilm reactors (Vocks et al., 2005), granular sludge systems (Qin et al., 2005) and suspend sludge systems (Coats et al., 2011; Winkler et al., 2011), and achieved well N and P removal performance. But most of them were carried out through complete nitrification-denitrification (NH<sub>4</sub><sup>+</sup>-N  $\rightarrow$  NO<sub>3</sub><sup>-</sup>-N  $\rightarrow$  N<sub>2</sub>) to achieve N removal from high C/N (>6) wastewaters (Coats et al., 2011; Vocks et al., 2005; Winkler et al., 2011). As for low C/N wastewater, a novel process, termed as PNEDPR (partial nitrification-endogenous denitrification and phosphorus removal), combined EBPR with partial nitrification and PED was developed for efficient nutrient removal. In particular, it is still not clear what conditions are favorable for a stable PNEDPR process, because PAOs, GAOs and DGAOs would compete for the limit carbon source. Besides, the aerobic duration and dissolved oxygen (DO) concentration would have significant effect on the concurrence of aerobic P uptake, partial nitrification and the intracellular carbons consumption, which might directly determine the subsequent PED performance.

This study aimed at developing a simple anaerobic/aerobic/anoxic (AOA) operated PNEDPR system using a sequencing batch reactor (SBR) to achieve deep-level nutrient removal from low C/N (~3) domestic wastewater without external carbon addition. To achieve the PNEDPR, anaerobic/aerobic/anoxic durations were appropriately selected based on an on-line control, and the sludge retention time (SRT) and aeration rate were timely regulated based on the nutrient removal performance. This study also investigated the nutrient removal pathways by determining and stiochiometries-based analyzing the variations of extracellular and intracellular substrates in a typical SBR operation cycle. Furthermore, the correlation of microbial population and activity with the nutrient and carbon removal was elucidated, and performance of the PNEDPR-SBR system was compared with other related systems to demonstrate its superiority.

#### 2. Methods

#### 2.1. PNEDPR-SBR system and experimental procedures

A SBR (working volume: 10 L) equipped with a pH/DO meter, an air diffuser and a mechanical stirrer was used as the PNEDPR-SBR system in this study (Fig. 1). The SBR was operated for 160 days at room temperature (22-26 °C) with the operating mode of 150 min anaerobic reaction (including 13 min feeding period), 180 min aerobic reaction, 990 min anoxic reaction (including 2 min sludge wasting, except for the first 15 days), 30 min settling, 5 min decanting and 25 min idling (Table 1). During the feeding period, 4 L domestic wastewater (composition described below) was added to the reactor at the beginning of anaerobic stage. During the sludge wasting period, 100 mL of mixed liquor was discharged at the end of the anoxic stage to achieve an aerobic solids retention time (SRT) of 12.5 d and a mixed liquor suspended solids concentration (MLSS) of  $3000 \pm 300 \,\text{mg/L}$ . The pH/DO meter was used to monitor the anaerobic/aeration/anoxic status, and to guide the regulation of aeration intensity in the aerobic stage. More detailed operation conditions of the SBR were shown in Table 1.

#### 2.2. Wastewater and seeding sludge

Domestic wastewater was taken from a septic tank in the residential area of Beijing University of Technology (Beijing, China). The main characteristics of the wastewater were: COD 160.4–224.8 mg/L, NH $^{+}_{4}$ -N 50.0–72.9 mg/L, NO $^{-}_{2}$ -N < 1 mg/L, NO $^{-}_{3}$ -N < 1 mg/L, PO $^{3}_{4}$ -P 3.6–9.3 mg/L, TN 58.8–73.5 mg/L, and C/N 2.7–3.8 (average 3.1). The activated sludge inoculated was taken from a pilot SBR system

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