



# Achieving simultaneous nitrogen removal of low C/N wastewater and external sludge reutilization in a sequencing batch reactor



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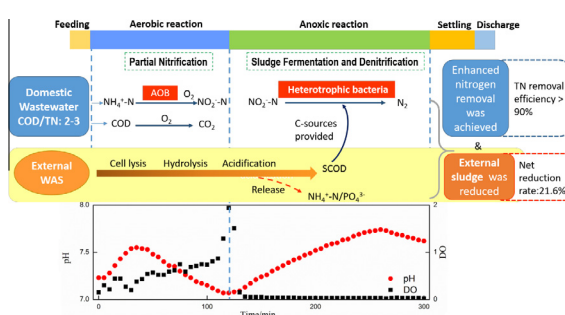
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## HIGHLIGHTS

- A novel PNSFD-SBR system was developed.
- Low C/N sewage BNR and external WAS reduction were achieved simultaneously.
- TN removal efficiency of 93% and WAS reduction rate of 22% were achieved.
- Micro-aeration maintained the PNSFD-SBR efficiency.
- The Illumina MiSeq analysis strengthened the understanding of the PNSFD system.

## GRAPHICAL ABSTRACT



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## ABSTRACT

An integrated partial nitrification, sludge fermentation and denitrification process in a sequencing batch reactor (PNSFD-SBR), was developed to enhance nitrogen removal from domestic wastewater with low C/N (i.e. SCOD/TN) between 2 to 3 and to reutilize/reduce external waste activated sludge (WAS). Average ammonium removal efficiency of 95.9%, total nitrogen removal efficiency of 93.5%, and external WAS reduction rate of 21.6% was achieved during long-term experiment at  $30 \pm 1^\circ\text{C}$ . Real-time pH-DO (dissolved oxygen) monitor and control played an important role in the PNSFD achievement. A batch test indicated that micro-aeration (less than 1 mg/L) sustained partial nitrification, which further facilitated subsequent sludge fermentation and denitrification processes. Moreover, Illumina MiSeq analysis demonstrated the predominant bacteria were *Rhodocyclaceae*, *Anaerolineaceae* and *Saprospiraceae* on family level and they were key to partial nitrification, denitrification and sludge fermentation, by which the feasibility of PNSFD process to achieve simultaneous nitrogen removal and external WAS reutilization/reduction was verified.

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## 1. Introduction

Due to severe environmental problems caused by excessive nitrogen in aquatic systems, biological nitrogen removal (BNR) from wastewater has drawn much more attention [1]. Intensive

aeration is required for the oxidation of ammonia into nitrate ( $4.3 \text{ g O}_2/\text{N}$ ) in nitrification and abundant organic substrates are needed for the reduction of nitrate to nitrogen gas in denitrification in traditional BNR processes [2]. For carbon-limited sewage in municipal wastewater treatment plants (WWTPs), how to remove nitrogen oxide has been a major challenge. One solution is to add external chemical organics (e.g. acetate) to supply carbon sources for denitrification, which has been widely applied in many WWTPs

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[3]. However, the huge purchase cost and unsustainable resource consumption restrict this application. The other solution is to apply novel processes to save carbon sources. For example, partial nitrification (ammonia is oxidized to nitrite) has been studied for decades, which can reduce the aeration consumption by 25% and save organic matters requirement by 40% for subsequent denitrification [4]. Several relative processes (e.g. single reactor for high ammonium removal over nitrite (SHARON), completely autotrophic nitrogen removal over nitrite (CANON), and oxygen-limited autotrophic nitrification denitrification (OLAND)) have been applied in treating high-ammonium wastewater [1]. Another process is simultaneous nitrification and denitrification (SND), which has been observed in numerous treatment systems by controlling oxygen input, and the low (0.5 mg/L) dissolved oxygen concentrations demonstrated significant SND [5].

With the wide application of conventional BNR processes, large amounts of waste activated sludge (WAS) are produced in WWTPs [6]. The expenditure on WAS treatment usually accounts for >60% of the total capital investment in WWTPs [7]. Many physicochemical techniques have been developed for sludge disintegration, including thermal, chemical, and advanced oxidation treatment [8], but these techniques may have negative impacts on environment [9]. Thus, developing a sustainable process capable of reutilizing WAS has become a new thinking to save costs and enhance treatment efficiency [10]. Previous studies have found that due to high content of organic compounds embodied in WAS, volatile fatty acids (VFAs) produced through hydrolysis and acidification were appropriate as internal carbon sources for BNR [11], which might substantially reduce the WWTP costs. The effect of pH, temperatures, and external sludge types on sludge digestion have been studied to optimize the yield of VFAs production [12]. Nevertheless, the traditional sludge digestion process shows two disadvantages: (i) the carbon sources absorbed in solid phase are difficult to elutriate [13], (ii) sludge retention time (SRT) of fermentation systems are shortened in order to avoid the VFAs consumption by methanogenic reaction, but a relative long SRT is required for thorough acidogenesis [14]. Thus, enhancing VFAs production from WAS digestion for in-situ denitrification can be a cost-effective and sustainable method to achieve simultaneous efficient BNR and external WAS reutilization/reduction when treating low C/N wastewater.

A novel process, namely integrated partial nitrification, sludge fermentation and denitrification in a sequencing batch reactor (termed as PNSFD-SBR), was proposed to treat domestic sewage with C/N less than 3 and reutilize/reduce external WAS. The main objectives of this study were: (1) investigate the start-up of PNSFD system and the BNR performance during 124-day operational period; (2) evaluate the on-line pH and DO control of the entire process; (3) study the impact of different DO levels on nitrogen removal performance; (4) identify the bacterial community of the sludge in PNSFD-SBR.

## 2. Materials and methods

### 2.1. Wastewater and sludge

The domestic wastewater used in this study was collected from the residential area of Beijing University of Technology. The fresh external WAS was taken from a pilot-scale SBR (working volume: 7 m<sup>3</sup>, SRT: 25 d), and this SBR was operated with nitrification-denitrification process. The characteristics of wastewater and external WAS are listed in Table 1. The seed sludge was obtained from a lab-scale SBR (working volume: 9 L, and operational parameters: Table S1) where simultaneous sludge fermentation and denitrification via nitrite took place. The seed sludge was concentrated before

**Table 1**  
Characteristics of wastewater and sludge.

| Parameter                       | Wastewater | External WAS  |
|---------------------------------|------------|---------------|
| pH                              | 7.3–7.8    | 7.5–7.8       |
| NH <sub>4</sub> <sup>+</sup> -N | 45–70      | –             |
| NO <sub>2</sub> <sup>-</sup> -N | 0–0.5      | –             |
| SCOD                            | 120–160    | 60–80         |
| SS                              | 118–132    | 10,000–15,000 |
| VSS                             | 62–76      | 7580–12,450   |

All values except for pH are expressed in mg/L. SCOD means the soluble COD.

added, and the mixed liquor suspended solids (MLSS) was 11,552 ± 319 mg/L and mixed liquor volatile suspended solids (MLVSS) was 9259 ± 62 mg/L, respectively.

### 2.2. PNSFD-SBR set-up and operation

An SBR (working volume: 2.5 L) was used for the PNSFD process (Fig. 1a) and operated in a temperature-controlled incubator (30 ± 1 °C). The SRT maintained at 39–42 d by controlling sludge wastage. At the start-up stage, 1 L of seed sludge was inoculated. Subsequently, wastewater and fresh WAS were injected into the reactor at the beginning of each cycle. Five periods were included in each cycle: 5 min feeding with 1.2–1.45 L sewage and 0.05–0.3 L external WAS, ~150 min aeration reaction with micro-aeration (lower than 1 mg/L) and magnetic mixing (700 rpm), ~200 min anoxic reaction with magnetic mixing, 60 min settling period, and 5 min discharging of 1.5 L supernatant (Fig. 1b). Magnetic stirring was conducted during aeration and anoxic periods. The reactor was in idle time in the rest of a cycle. According to the turning point or sudden change of DO and pH profiles, the end of partial nitrification and denitrification were determined, and thereby the mixed liquor samples were collected. As shown in Table 2, the entire PNSFD-SBR operation was divided into three Phases according to the mass of sludge consumption. Although the mass of injected sludge ( $M_{\text{inf}}$ ) and wasted sludge ( $M_{\text{eff}}$ ) varied, the amounts of sludge consumption ( $\Delta M$ ) increased gradually.

In order to further elucidate the effect of DO concentrations on the PNSFD process, a batch test was conducted in three SBRs (working volume: 1 L, DO low: <1 mg/L, moderate: 2–3 mg/L, high: >4 mg/L) individually with the operation mode the same as in the PNSFD-SBR. The external sludge in the batch tests was the same as in PNSFD-SBR.

### 2.3. Analytical methods

The liquid samples were collected from the PNSFD-SBR system and filtered through medium speed filter papers (pore size: 30–50 µm). The concentrations of NH<sub>4</sub><sup>+</sup>, NO<sub>2</sub><sup>-</sup>, and NO<sub>3</sub><sup>-</sup> were analyzed using an automatic flow injection analyzer (QuikChem8500 Series2, Lachat Company, USA). The concentrations of chemical oxygen demand (COD), SS, and VSS were measured according to standard methods [15]. The temperature, pH, and DO were monitored using WTW 340i probes (WTW Company, Germany). Soluble polysaccharide was measured using the phenol-sulfuric method with glucose as the standard and soluble protein was determined using the Lowry-Folin method with BSA as the standard [16]. An Agilent 7890A gas chromatography (GC) was used for the quantification of VFAs. The filtrate was acidified with 10% H<sub>3</sub>PO<sub>4</sub> to about pH 4.0, and collected in a 2.0 mL gas chromatography vial before assaying on the GC with flame ionization detector (FID) and the automatic liquid sampler.

In order to analyze the microbial communities of PNSFD-SBR, the biomass sample was collected from the system on Cycle 80 with stable nitrogen removal performance. The genomic DNA

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