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RESEARCH ARTICLE

# Enhanced nutrients removal from municipal wastewater through biological phosphorus removal followed by partial nitritation/anammox

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## HIGHLIGHTS

- EBPR and PN/A were combined to enhance nutrients removal from municipal wastewater.
- High effluent quality of 0.25 mg $TP \cdot L^{-1}$ and 10.8 mg $TN \cdot L^{-1}$ was obtained.
- Phosphorus and nitrogen removal was achieved in two separated units.
- A proper post-anoxic phase improved the nitrogen removal performance of PN/A unit.

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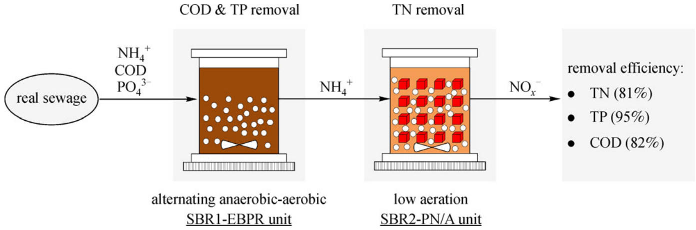
## GRAPHIC ABSTRACT



## ABSTRACT

Conventional biological removal of nitrogen and phosphorus is usually limited due to the lack of biodegradable carbon source, therefore, new methods are needed. In this study, a new alternative consisting of enhanced biological phosphorus removal (EBPR) followed by partial nitritation-anammox (PN/A), is proposed to enhance nutrients removal from municipal wastewater. Research was carried out in a laboratory-scale system of combined two sequencing batch reactors (SBRs). In SBR1, phosphorus removal was achieved under an alternating anaerobic-aerobic condition and ammonium concentration stayed the same since nitrifiers were washed out from the reactor under short sludge retention time of 2–3 d. The remaining ammonium was further treated in SBR2 where PN/A was established by inoculation. A maximum of nitrogen removal rate of 0.12 kg $N \cdot m^{-3} \cdot d^{-1}$ was finally achieved. During the stable period, effluent concentrations of total phosphorus and total nitrogen were 0.25 and 10.8 $mg \cdot L^{-1}$, respectively. This study suggests EBPR-PN/A process is feasible to enhance nutrients removal from municipal wastewater of low influent carbon source.

## 1 Introduction

Nowadays, biological phosphorus and nitrogen removal system has been widely demonstrated in municipal wastewater treatment. For biological treatment process, influent carbon source plays an important role since it is not only taken up by polyphosphate accumulating organisms (PAO) for phosphate removal, but also used as electron donor by denitrifies to remove nitrogen. When influent carbon source is insufficient, the performance of biological nutrients removal would be serious limited [1]. For municipal wastewater of low chemical oxygen demand (COD)/N ratio, several strategies have been established to improve the nutrient removal efficiency, such as developing alternative carbon source and improving the utilization ratio of influent carbon source [2–5]. In addition, novel systems requiring less carbon source have been integrated

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into municipal wastewater treatment, such as partial nitrification-denitrification process, denitrification via PAO and partial nitritation-anammox (PN/A) process [6–10]. Among these novel systems, PN/A process is currently raising attention and considered as a new pathway to improve nutrient removal of municipal wastewater [11]. PN/A process is a complete autotrophic process and requires no carbon source for ammonium removal. When it is involved in municipal wastewater treatment, the competition of organic substrate between enhanced biological phosphorus removal (EBPR) and denitrification could be avoided and the improvement of simultaneous removal of nitrogen and phosphorus is expected. Thus, EBPR integrated with PN/A would be a promising alternative to improve nutrient removal from municipal wastewater, especially when influent carbon source is limited.

Although the combination of EBPR and PN/A has several advantages, it is still challengeable for its application in municipal wastewater treatment. One of challenges is maintaining stable operation of PN/A process under low ammonium concentration and temperature [12]. Municipal wastewater PN/A process has been established using synthetic wastewater [13–15], or pre-treated municipal wastewater [16–18], but system performance such as effluent quality and operational stability still requires further improvement [16,19,20]. Besides, research on combing EBPR and PN/A is still rare, and its status as an alternative to conventional nutrients removal process remains unclear, especially in the actual municipal wastewater treatment.

In this study, a combined system consisting of two sequencing batch reactors (SBRs) was established to treat actual municipal wastewater via integration of EBPR and PN/A. Raw wastewater was first fed to SBR1 where phosphorus was removed through EBPR process. Then the treated wastewater was fed to SBR2 to remove ammonium through PN/A process. Main objectives of this study were to investigate the nutrient removal performance of the combined system and evaluate its potential in municipal wastewater treatment.

## 2 Materials and methods

### 2.1 Reactor set up

A schematic diagram of the integrated system was shown in Fig. 1. The system was operated for two cycles every day and the whole experiment lasted approximately 5 months. Both reactors had a working volume of 120 L. An air compressor (SW550A, Blue air company, Germany) was used for aeration through the fine bubble aerator stalled at the bottom of the reactors. A mechanical stirrer (RW20, IKA Company, Germany) was used for anaerobic mixture of the SBR1. A nitrogen gas producer (KPS-N-16, SuLong Company, China) was used for the mixture of SBR2 in anoxic phase. The concentration of dissolved oxygen (DO) and temperature were measured by oxygen and temperature probes (WTW 340i, WTW Company, Germany).

### 2.2 Wastewater and seed sludge

The municipal wastewater used in this study was collected from the primary settle tank of GaoBeiDian wastewater treatment plant (WWTP) in Beijing, China. Raw wastewater was continuously piped from the primary settle tanks to the influent storage tank. Main characteristics of the municipal wastewater were as follows: COD = 160–320 mg$\cdot$L$^{-1}$, total nitrogen (TN) = 42–67 mg$\cdot$L$^{-1}$, soluble chemical oxygen demand (SCOD) = 120–180 mg$\cdot$L$^{-1}$, $NH_4^+$-N = 36–58 mg$\cdot$L$^{-1}$, $NO_2^-$-N = 0–0.3 mg$\cdot$L$^{-1}$, $NO_3^-$-N = 0–1.1 mg$\cdot$L$^{-1}$, total phosphorus (TP) = 3.0–8.7 mg$\cdot$L$^{-1}$, suspended solids (SS) = 74–110 mg$\cdot$L$^{-1}$, the temperature was within 16°C–24°C.

SBR1 was inoculated with the return activated sludge collected from GaoBeiDian WWTP. The initial concentration of the mixed liquors suspended solid (MLSS) was 4000 mg$\cdot$L$^{-1}$. SBR2 was inoculated with activated sludge and anammox biofilm carrier collected from a full-scale PN/A reactor for side stream treatment of GaoBeiDian WWTP [21]. After inoculation, the initial MLSS concentration of activated sludge in the SBR2 was 3500 mg$\cdot$L$^{-1}$.

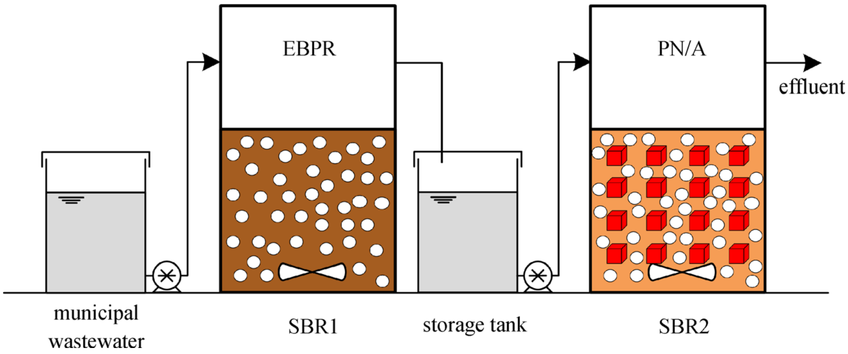

**Fig. 1** Schematic diagram of the EBPR-PN/A process

The volume ratio of biofilm carrier was 12%. The initial MLSS concentration of biofilm was 4300 mg·L$^{-1}$.

### 2.3 Experimental procedure

During each cycle, SBR1 was fed with 60 L wastewater, followed by 30 min anaerobic mixture and 90 min aeration. The concentration of DO during aerobic phase was not controlled but remained over 3 mg·L$^{-1}$. The supernatant of SBR 1 was discharged after a 20 min settling time. The sludge retention time (SRT) of SBR1 was controlled at 2–3 d by discharging 3.3–5 L mixed liquor at the end of aerobic phase.

The experiment of the SBR2 was divided into two phases according to the operating conditions. In phase I (1–100 d), SBR2 was continuously aerated for various duration (1.6–6 h) with DO concentration of 0.1–0.2 mg·L$^{-1}$. In phase II (101–151 d), SBR2 operation comprised an aerobic reaction of 3–3.5 h, followed by an anoxic reaction of 1 h. The DO concentration of the aerobic reaction was within 0.3–0.4 mg·L$^{-1}$. After 1 h settling, the supernatant of the SBR2 was discharged as final effluent. The SRT of SBR2 was not manually controlled. Except for the suspended solid in effluent, no sludge was discharged from SBR2.

### 2.4 Chemical analysis

The concentrations of COD, SCOD, TN, $NH_4^+$-N, $NO_2^-$-N, $NO_3^-$-N, TP, SS and MLSS were measured according to standard methods [22]. All the water samples were filtered with a 0.45 µm filter prior to analyses.

## 3 Results and discussion

### 3.1 Enhanced phosphorus removal in SBR1

Municipal wastewater was first fed to SBR1 from the storage tank. SBR1 was operated under alternating anaerobic and aerobic conditions with a bulk DO concentration of 3 mg·L$^{-1}$ in aerobic phase and SRT of 2–3 d. As shown in Fig. 2, despite of the variations of temperature (16°C–22°C) and influent TP (3.0–8.7 mg·L$^{-1}$), biological phosphorus removal was well achieved with a removal efficiency of 95% and an effluent TP concentration of 0.25 mg·L$^{-1}$. Results of the continuous operation indicated that the effluent phosphorus could meet the Chinese National First A-level Sewage Discharge Standard when the EBPR process reached steady-state.

Nitrification was negligible in SBR1, as revealed by the similar ammonium concentration in influent (47.3 mg·L$^{-1}$) and effluent (46.6 mg·L$^{-1}$) and confirmed by the negligible nitrate and nitrite in effluent. The average removal efficiency of TN was only 9%. On the other hand, the concentration of SCOD was reduced to 48–65 mg·L$^{-1}$ with an average removal efficiency of 62% and the SCOD/N ratio of the effluent was 1–1.3. It has been reported that a SCOD/N ratio below 1.4 is desirable for efficient PN/A process [23]. Thus, SBR1 provided a suitable feed for PN/A process.

In traditional biological nutrient removal system, both denitrification and phosphorus removal require organic carbon sources. The removal efficiency of TP was often limited when the organic carbon in wastewater was

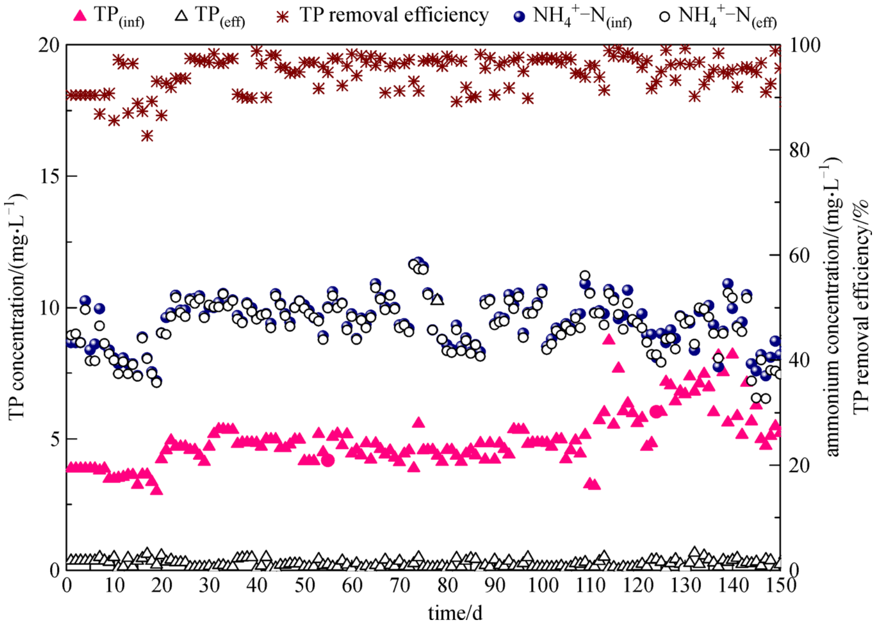

**Fig. 2** Nutrient removal performance of SBR1 (EBPR unit): variations of influent TP, influent ammonium, effluent TP, effluent ammonium and TP removal efficiency

insufficient. In this study, the initiation of nitrification was prevented in SBR1 by controlling an appropriately low SRT (2–3 d), thereby denitrification would not occur. In this case, the SCOD available for phosphorus removal was sufficient and high phosphorus removal efficiency was achieved.

### 3.2 Nitrogen removal performance of PN/A process

The operation of SBR2 was divided into two phases. In phase I (1–100 d), SBR2 was inoculated with anammox biofilm and operated with a bulk DO concentration of 0.1–0.2 $mg \cdot L^{-1}$. In the operational period of 1–14 d, the aeration time of each cycle in SBR2 increased from 1.6 to 4 h. As shown in Fig. 3, both effluent ammonium and nitrite concentration progressively decreased, resulting in an effluent TN of 11.5 $mg \cdot L^{-1}$. During the following period of 15–50 d, aeration time was kept at 4 h and a maximum nitrogen removal rate of 0.12 $kg\ N \cdot m^{-3} \cdot d^{-1}$ was achieved with a removal efficiency of 77%, indicating the establishment of PN/A process. During the operational period of 51–100 d, temperature gradually decreased from 22°C to 16°C. As shown in Fig. 2, effluent ammonium began to increase when temperature decreased, reaching over 20 $mg \cdot L^{-1}$ on 70 d. To improve the nitrogen removal efficiency, the aeration time of SBR2 was extended to 5–5.5 h. Consequently, the effluent ammonium decreased to lower than 10 $mg \cdot L^{-1}$ in two weeks and the nitrogen removal rate was reduced to 0.08 $kg\ N \cdot m^{-3} \cdot d^{-1}$.

It has been reported that PN/A process was sensitive to the variation of temperature. Lotti et al. [14] found that PN/A operation at 10°C caused a decrease in anammox activity and process efficiency. Similarly, Gilbert et al. [13] reported a significant decrease of PN/A system performance under low temperature. Besides, in most studies, anammox bacteria was more sensitive to the low temperature and the effluent nitrite was therefore significantly accumulated [13,14,24]. In this study, however, the increase of effluent ammonium was observed at low temperature and nitrite was not significantly accumulated (Fig. 3). It indicated that nitritation activity was more sensitive to low temperature, which was inconsistent to previous reports [13,14,24]. Besides the low temperature, the gradual washout of the suspended activated sludge (from 2520 $mg \cdot L^{-1}$ on Day 51 to 1640 $mg \cdot L^{-1}$ on Day 100) might also contribute to the decay of nitration in present study. It is reported that even a small level of washout of suspended sludge might induce the decrease of nitritation activity of the combined PN/A system since ammonium-oxidizing bacteria was mainly located in the suspended sludge [21,25].

In phase II of SBR2 (101–151 d), the effect of post anoxic reaction on PN/A system was investigated. SBR2 was first aerated for 3–3.5 h after feed, followed by an anoxic period of 1 h. As shown in Fig. 3, effluent TN of 8.6 $mg \cdot L^{-1}$ was obtained in phase II with a nitrogen removal efficiency of 81%. Experimental results indicated post anoxic phase could improve PN/A performance.

The addition of post anoxic period triggered a simultaneous decrease of effluent ammonium, nitrite and nitrate as compared to phase I, and therefore improved the nitrogen removal efficiency of PN/A (Table 1). The reduction of nitrate indicated the occurrence of denitrification in the post anoxic period. Besides, denitrification (nitrate→ nitrite) could be integrated with anammox (nitrite + ammonium→ $N_2$) in the anoxic condition, which further improved the nitrogen removal efficiency [26]. In this study, most carbon sources have been removed in aerobic period, denitrification in post anoxic period might be achieved by using internal carbon sources [3].

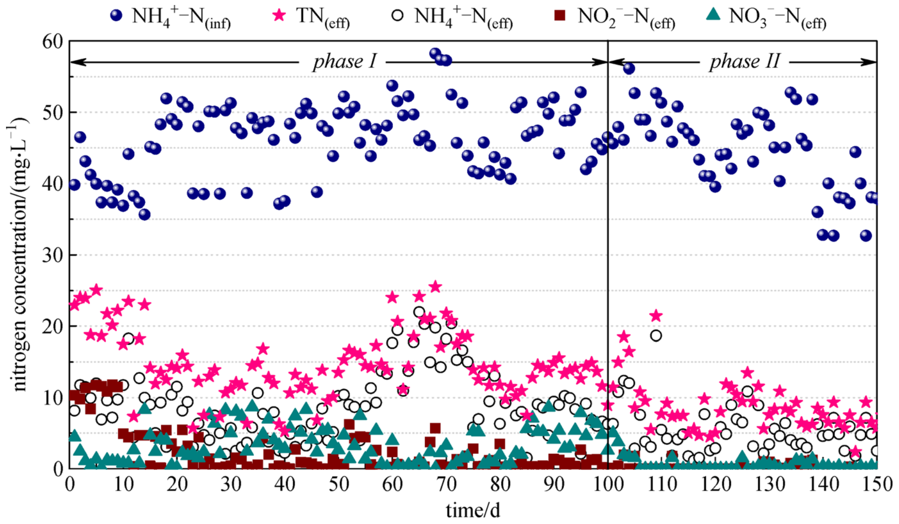

**Fig. 3** Nitrogen removal performance of SBR2 (PN/A unit): variations of influent ammonium, and effluent ammonium, nitrite, nitrate and TN

**Table 1** Summary of system performance of PN/A reactor in phase I and II

| phase | phase /d | effluent concentration /(mg·L$^{-1}$) | | | nitrogen removal efficiency /% |
|---|---|---|---|---|---|
| | | $NH_4^+$-N | $NO_2^-$-N | $NO_3^-$-N | |
| I | 1–100 | 9.0 | 2.3 | 3.4 | 68 |
| II | 101–150 | 5.3 | 0.3 | 0.5 | 81 |

Additionally, post anoxic phase reduced the residual nitrite concentration. Sludge floatation in the settling phase due to anammox or denitrification could be avoided and effluent biomass washout in discharge phase would be prevented, which favors the process stability [27]. Overall, post-denitrification appeared as an approach to improve nitrogen removal of PN/A process.

### 3.3 Overall performance of the combined system in municipal wastewater treatment

In this study, the integrated process of EBPR and PN/A presented good nutrients removal performance of the actual municipal wastewater. The average TP, ammonium and TN in effluent of the integrated system were 0.25, 5.8 and 10.8 mg·L$^{-1}$, respectively. Effluent phosphorus could directly meet the discharge standard without the chemical precipitation. Besides, without adding external carbon source, a maximum nitrogen removal rate of 0.12 kg N·m$^{-3}$·d$^{-1}$ was obtained with a nitrogen removal efficiency of 68%–81%.

This study shows the integrated system is favorable to treat municipal wastewater of a low influent COD/N ratio but it requires more research before its application. In this study, stable and efficient phosphorus removal was achieved in the EBPR stage, while both nitrogen removal performance and system stability of PN/A stage require further improvement. First of all, effluent ammonium concentration of the PN/A reactor failed to meet the discharge standard. A polishing unit could be involved and the optimization of control strategies would be useful. On the other hand, not only the performance but also the stability of the PN/A reactor under low temperature requires further investigation. It has been reported that low working temperature not only limited the growth and activity of ammonium-oxidizing bacteria (AOB) and anammox bacteria, but also induced the proliferation of nitrite-oxidizing bacteria (NOB) [24]. Finally, an assessment should be made of recovering phosphorus and generating energy from the waste sludge of EBPR process.

## 4 Conclusions

Simultaneous removal of organic carbon, phosphorus and ammonium from municipal wastewater via combination of EBPR and PN/A process were successfully achieved. The combined system was able to efficiently remove nitrogen and phosphorus from municipal wastewater, resulting in the effluent concentration less than 10.8 and 0.25 mg·L$^{-1}$, respectively. EBPR was stable despite of the temperature variations but PN/A performance was impacted by the low temperature due to the decrease of nitritation activity. Municipal PN/A process could be improved by the addition of appropriate post-anoxic phase.

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