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APPROPRIATE TECHNOLOGIES TO COMBAT WATER POLLUTION

Performance and recovery of a completely separated partial nitritation and anammox process treating phenol-containing wastewater

Wei Wang[1,2] · Chao Pang[1] · Julian Muñoz Sierra[3,4] · Zhenhu Hu[1] · Xuesong Ren[1]

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Abstract

Anammox process is considered as a promising technology for removing total nitrogen from low-strength ammonium and phenol-containing wastewater. However, it is still a challenge for the anammox process to treat high-strength ammonium and phenol-containing wastewater. A completely separated partial nitritation and anammox (CSPN/A) process was developed to remove total nitrogen from high-strength phenol-containing wastewater. About 92% of COD, 100% of phenol, and 82.4% of total nitrogen were successfully removed at a NH_4^+-N concentration of 200 mg L^{-1} with a phenol/NH_4^+-N mass ratio of 0.5 in the CSPN/A process. Furthermore, a shock loading of 300 mg phenol L^{-1} with a phenol/NH_4^+-N mass ratio of 1.5 led to a complete failure of partial nitritation, but the performance was rapidly recovered by the increase of NH_4^+-N concentration. Although the activities of ammonium-oxidizing bacteria and anammox bacteria were severely inhibited at a phenol/NH_4^+-N mass ratio of 1.5, the enrichment of efficient phenol degraders in the CSPN stage could strengthen the performance robustness of partial nitritation and anammox process. Therefore, this study presented a new insight on the feasibility of the anammox process for treating high-strength ammonium and phenol-containing wastewater.

Keywords Partial nitritation · Anammox process · Phenol · Ammonium-oxidizing bacteria · Performance robustness · Phenol degraders

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✉ Wei Wang
wang_wei@hfut.edu.cn; dwhit@126.com

[1] Department of Municipal Engineering, School of Civil and Hydraulic Engineering, Hefei University of Technology, Hefei 230009, China

[2] Key Laboratory of Urban Pollutant Conversion, Chinese Academy of Sciences, University of Science and Technology of China, Hefei 230009, China

[3] Section Sanitary Engineering, Department of Water Management, Delft University of Technology, Stevinweg 1, 2628CN Delft, The Netherlands

[4] KWR Watercycle Research Institute, Groningenhaven 7, 3430BB Nieuwegein, The Netherlands

Introduction

Phenol and ammonium were identified as the two major compounds in the coal gasification wastewater (Gai et al. 2008; Zhuang et al. 2016). Conventional biological nitrogen removal process not only consumed a lot of energy (Ma et al. 2016) but also was susceptible to the toxicity of phenol (Lauchnor and Semprini 2013). The partial nitritation/anammox (PN/A) process was considered as one of the most feasible methods for treating ammonium-containing wastewater (Li et al. 2017). Currently, almost 100 full-scale PN/A installations were in operation or under construction/planning in the world (Lackner et al. 2014). The anammox process was suitable to remove nitrogen from low-strength phenol-containing wastewaters and not necessarily to remove nitrogen from wastewaters containing high-strength phenol. The adverse effect of phenol on the anammox activity was observed when the concentration was less than 200 mg phenol L^{-1} (Yang et al. 2013). Subsequently, the anammox activity was severely suppressed

when the phenol concentration was about 300 mg L^{-1} (Pereira et al. 2014). Therefore, the anammox technology was a promising strategy for efficiently removing nitrogen from phenol-containing wastewater, but a stable partial nitritation stage prior to the anammox reaction was essential to treat high-strength phenol-containing wastewater (Li et al. 2011b).

Because the ammonium-oxidizing bacteria (AOB), nitrite-oxidizing bacteria (NOB), and anammox bacteria were easily affected by the changes of environmental conditions (Liu et al. 2017b), the partial nitritation and anammox process for treating high-strength phenol-containing wastewater was challenging to control. Firstly, an enrichment of AOB could be established by the suppression of NOB, but the tolerance of AOB and NOB to phenol toxicity might differ (Gao et al. 2017). Secondly, there were some potential inhibitory factors on the performance of the anammox process treating phenol-containing wastewater, such as high sensitivity of anammox microorganisms to environment conditions (Jin et al. 2013; Pereira et al. 2014). Although the anammox bacteria could adapt over an extended period to phenol-containing wastewater at low phenol/NH_4^+-N mass ratio (Toh and Ashbolt 2002), the performance robustness was weakened by the unstable partial nitritation. Therefore, a more stable and controllable environment for AOB and anammox bacteria in the phenol-containing wastewater treatment process was critical.

This study aimed to develop a completely separated partial nitritation and anammox (CSPN/A) process to remove nitrogen from phenol-containing wastewater. Firstly, the effect of phenol/nitrogen (phenol/N) mass ratio on the activities of AOB, NOB, and specific anammox activity (SAA) was investigated. Furthermore, the performance and recovery of CSPN/A process treating phenol-containing wastewater were evaluated. Finally, the analysis of the microbial community structure and activities in the CSPN/A process were correspondingly discussed.

Materials and methods

Experimental setup and operation

The schematic diagram of the experimental setup is shown in Fig. 1. A laboratory-scale CSPN/A system was made up of two reactors, one continuous membrane bioreactor (MBR) for completely separated partial nitritation (CSPN) and a sequencing batch reactor (SBR) for the anammox reaction. The CSPN reactor was composed of a cylindrical reactor with a working volume of 2.5 L and a submerged membrane module with a nominal pore size of 0.2 μm and surface area of 0.21 m^2. The CSPN reactor was operated at a dissolved oxygen (DO) of 0.5 mg L^{-1} and pH of 8.0. The temperature and hydraulic retention time (HRT) in the CSPN reactor were set at 35 ± 2 °C and 24 h, respectively. The working volume of the SBR was 1.6 L. The effluent of CSPN reactor was stripped with nitrogen gas in a sealed container with an exhaust pipe at the top. After flushing with nitrogen gas, the solution pH was adjusted to 6.8–7.0. The final concentration of ammonium, nitrite, nitrate, and phenol were measured as the influent quality of anammox stage. Subsequently, the effluent was pumped into the SBR which was operated at a temperature of 35 °C and a HRT of 17.3 h.

The operation of CSPN/A process was divided into three phases. During the start-up period (phase I, 1–125 days), the influent concentrations of NH_4^+-N and phenol were 200 and 100 mg L^{-1}, respectively. During the phenol shock period (phase II, 137–161 days), the influent phenol concentration was increased to 300 mg L^{-1} in the CSPN reactor. During the recovery period (phase III, 172–206 days), the influent concentration of NH_4^+-N was increased to 600 mg L^{-1} in the CSPN reactor, but the effluent of CSPN reactor was equally split into two streams. One stream was pumped into the initial anammox reactor. The other stream was pumped into a control anammox reactor that was not exposed to phenol.

Inoculum and synthetic wastewater

The inoculum of the CSPN reactor was activated sludge obtained from a full-scale municipal wastewater treatment plant (SBR process, treatment capacity 50,000 t day^{-1}) in Hefei, China. The concentrations of mixed liquid suspended solids (MLSS) and mixed liquid volatile suspended solids (MLVSS) were 4.6 and 2 g L^{-1}, respectively, in the CSPN reactor. The inoculated sludge of the SBR was collected from a high-rate anammox reactor with a maximum nitrogen removal rate of 0.36 kg N m^{-3} day^{-1}. The seed sludge was brick red anammox granular sludge with SAA of 73.20 ± 1.04 mg NH_4^+-N g^{-1} VSS day^{-1}. The dominant anammox genus of sludge was *Candidatus Kuenenia* with a relative abundance of 39.31%. The inoculum concentrations of the SBR were 4.86 g MLSS L^{-1} and 2 g MLVSS L^{-1}, respectively. The CSPN reactor was fed with the wastewater containing (mg L^{-1}) NH_4Cl (382–2293), phenol (100–300), $MgSO_4{\cdot}7H_2O$ (300), $KHCO_3$ (400–600), $CaCl_2{\cdot}2H_2O$ (180), KH_2PO_4 (25), and 1 mL of micronutrients I and II. The composition of micronutrients I and II was previously described by Yang and Jin (2012).

The effect of phenol/N ratio on the activities of AOB, NOB, and SAA

The effect of phenol/NH_4^+-N mass ratio on the maximum NH_4^+-N depletion rates of the activated sludge that was not

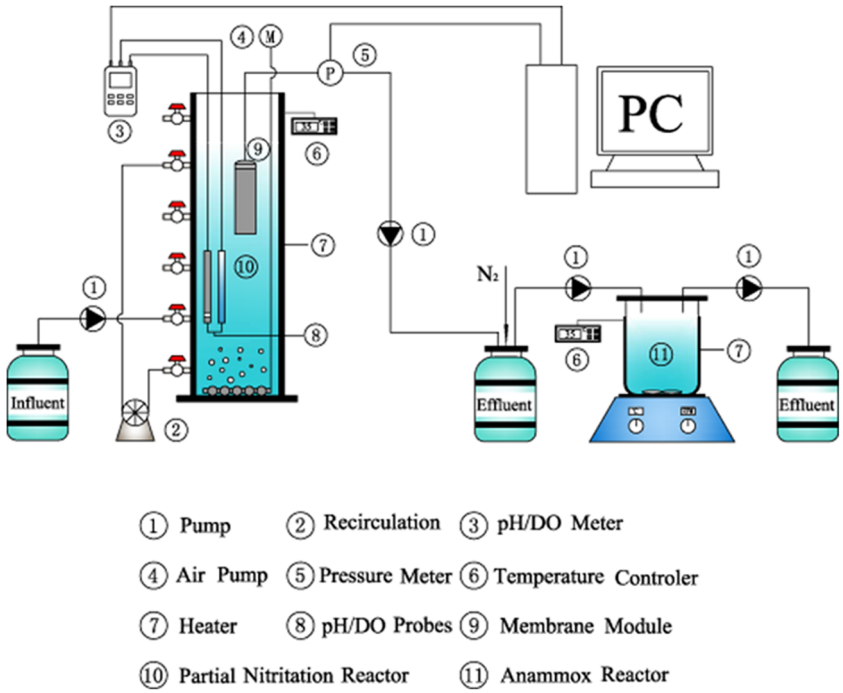

Fig. 1 Schematic diagram of experimental setup

acclimated with phenol was assessed in batch assays. Phenol concentrations of 0, 5, 10, 15, and 100 mg L^{-1} were respectively dosed to the beakers filled with activated sludge of 1.5 g VSS L^{-1} and incubated at 30 °C in a shaker. The activities of AOB were calculated based on the NH_4^+-N depletion rate (mg NH_4^+-N g^{-1} VSS day^{-1}). Similarly, the shock of phenol/NO_2^--N mass ratio on the maximum NO_2^--N depletion rate of the activated sludge was also elucidated. The concentrations of NO_2^--N and activated sludge were set at 15 mg L^{-1} and 1.5 g VSS L^{-1} in the beakers with phenol concentrations of 0, 5, 10, 15, and 100 mg L^{-1}, respectively. The activity of NOB was measured by NO_2^--N depletion rate (mg NO_2^--N g VSS^{-1} day^{-1}). The effect of phenol/NH_4^+-N mass ratio on the specific anammox activity (SAA) of non-acclimated to phenol sludge was operated using 250 mL serum bottles with sludge concentration of 1.5 g VSS L^{-1}. Both the concentrations of NH_4^+-N and NO_2^--N were initially controlled at 50 mg L^{-1}. The serum bottles were incubated with 0, 20, 50, and 100 mg phenol L^{-1}, respectively. The mixing rate and temperature were controlled at 150 rpm and 35 °C, respectively, and all tests were performed in duplicate. The NH_4^+-N depletion rate was assessed to calculate the SAA of the sludge and was expressed as mg NH_4^+-N g VSS^{-1} day^{-1}. The activity tests were carried out as previous studies (Dapena-Mora et al. 2004; Tang et al. 2009; Wu et al. 2016).

Phenol utilization rate of sludge

The substrate utilization rate (SUR) of phenol was used to evaluate the phenol depletion rate of the sludge in the CSPN reactor. The concentration of phenol was controlled at 20 mg L^{-1} with a mass ratio of phenol to sludge of 1:40. The details of SUR tests were carried out as described by Wang et al. (2017). All SUR tests were performed in triplicate.

Other analytical methods and statistical data analysis

The concentrations of COD, NO_3^--N, NO_2^-N, NH_4^+-N, MLSS, and MLVSS were determined according to the Standard Methods (APHA 2005). The activities of AOB, NOB, and SUR of sludge taken from the CSPN reactor and SAA of sludge taken from the anammox reactor were also detected in the end of each phase. The concentration of phenol was monitored following the method described in Wang et al. (2017), with the high-performance liquid chromatography system (1260 Infinity; Agilent Inc., USA). The sludge taken from the CSPN reactor (MBR) was used to analyze the microbial community structure. The PCR primers used were V4 universal primers (515F: GTGCCAGCMGCCGCGGTAA and 806R: GGACTACHVGGGTWTCTAAT). The high-throughput sequencing was performed using the Illumina Miseq platform (Illumina, Inc., San Diego, CA, USA). All analysis workflow was performed by Sangon Biotech Co., Ltd. (Sangon, China). The effect of phenol/N mass ratio on the sludge activity among the test and control groups, including the activities of AOB, NOB, and SAA of sludge, was statistically assessed by a one-way ANOVA method using Origin version 9.1 (OriginLab Corporation, USA).

Results and discussion

Effect of phenol/N mass ratios on the activities of AOB, NOB, and SAA of sludge

The effect of phenol/N mass ratio on the activities of AOB, NOB, and SAA of sludge are shown in Fig. 2. The results indicated that the activity of AOB decreased from 115 (control group) to 35 mg NH_4^+-N g^{-1} VSS day^{-1} at a phenol/N ratio of 0.17. Furthermore, it was completely inhibited by the increase of phenol/NH_4^+-N mass ratio from 0.33 to 1.67. The phenol/NH_4^+-N mass ratio had a

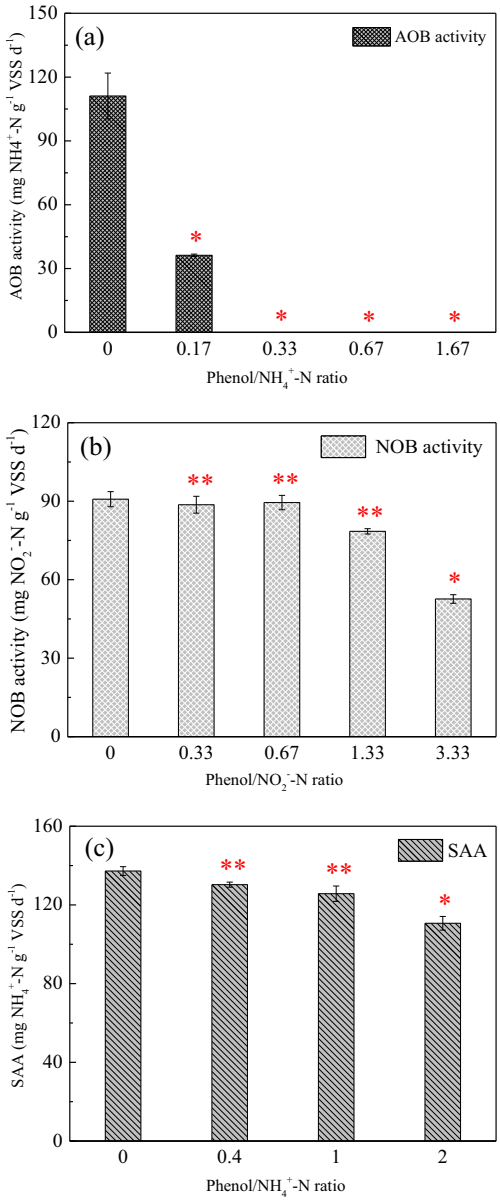

Fig. 2 Effect of phenol/N ratio on the activities of AOB, NOB, and SAA of sludge (*the difference between test and control group is significant at the 0.05 level; **the difference between the test and control group is not significant at the 0.05 level)

significant impact on the activity of AOB ($p = 0.0232$, < 0.05). For the NOB, the bacterial activity showed a better tolerance to phenol than the AOB. The activities of NOB were around 90 mg NO_2^--N g^{-1} VSS day^{-1} in the control group. There was no evident decrease of NOB activity when the phenol/NO_2^--N mass ratio was increased from 0 to 0.67 ($p = 0.7771$, > 0.05). Moreover, the activity of NOB was gradually decreased to around 80 and 55 mg NO_2^--N g^{-1} VSS day^{-1} at a phenol/NO_2^--N mass ratio of 1.33 and 3.33, respectively. No statistically significant difference of NOB activity was observed between phenol/NO_2^--N mass ratio at 0 and 1.33 ($p = 0.0573$, > 0.05), while the difference between the ratios of 0 and 3.33 was significant ($p = 0.0075$, < 0.05). Therefore, the partial nitration was easily inhibited because the AOB was more sensitive to the toxicity of phenol than NOB. Liu et al. (2005) reported that 20 mg L^{-1} phenol decreased the SUR of AOB by half while the SUR of NOB was only decreased by 10%. As demonstrated by Guo et al. (2017), AOB was more sensitive to phenol stress than NOB due to a DO concentration imbalance in the bulk liquid. Phenol would reduce the NH_4^+-N oxidation rate, and the consumption of nitrite was not affected (Toh and Ashbolt 2002). For the anammox bacteria, the maximum SAA of 140 mg NH_4^+-N g^{-1} VSS day^{-1} was obtained at the control group. When the phenol/NH_4^+-N mass ratio was increased from 0 to 2, the SAA of sludge was reduced by 20% as compared to the control group and remained at 100 mg NH_4^+-N g^{-1} VSS d^{-1}. There was no significant difference of SAA of sludge between the phenol/NH_4^+-N mass ratio of 0 and 1 ($p = 0.1243$, > 0.05). The SAA of sludge was decreased to 100 mg NH_4^+-N g^{-1} VSS day^{-1} at the phenol/NH_4^+-N mass ratio of 2, which was significant when compared to the SAA of sludge at the control group ($p = 0.0232$, < 0.05). The batch test performed by Yang et al. (2013) showed that 672 mg L^{-1} phenol brought out 50% inhibition on anammox. The inhibition of anaerobic ammonia oxidation by phenol might be attributed to the phenol toxicity which had significant impact on cell membrane, improving the cell membrane permeability (Beristain-Cardoso et al. 2009). However, the tolerance of anammox bacteria to phenol was higher than the AOB and NOB, which indicated that the anammox process was feasible to be applied in phenol-containing wastewater treatment. The control of partial nitritation was the key step for treating phenol-containing wastewater by anammox treatment, which also played an important role in the reduction of phenol concentration and decline of phenol/NH_4^+-N mass ratio at the anammox stage. In order to reduce the toxicity of phenol on the AOB, the aeration and/or dilution methods could be used while the microbial community structure of anammox process was hampered (Lauchnor and Semprini 2013; Li et al. 2011a; Miao et

al. 2018). Therefore, the complete separation of partial nitritation and anammox reaction might be favorable for controlling the different microbial structure dynamics against phenol toxicity.

Long-term operation of CSPN/A process at a phenol/NH_4^+-N mass ratio of 0.5 (phase I)

Variations of NH_4^+-N, NO_2^--N, NO_3^--N, and COD concentration in the CSPN/A process at phase I are shown in Fig. 3. Correspondingly, the average removal efficiencies of NH_4^+, phenol, COD, and TN in the CSPN/A system are shown in Table S1. The influent concentrations of NH_4^+-N and phenol were maintained at 200 and 100 mg L^{-1}, respectively, with corresponding nitrogen loading rate of 0.2 kg N m^{-3} day^{-1} and phenol loading rate of 0.1 kg m^{-3} day^{-1}. In the CSPN reactor, the effluent concentrations of NH_4^+-N, NO_2^--N, and COD were about 94.08 ± 24.61, 80.24 ± 23.22, and 105.07 ± 36.26 mg L^{-1}, respectively, from days 15 to 25. Since day 28, the effluent of CSPN reactor was treated by the anammox reactor. From days 115 to 125, the effluent concentrations of NH_4^+-N, NO_2^--N, and COD were about 80.44 ± 8.80, 79.38 ± 12.38, and 12.04 ± 40.44 mg L^{-1}, respectively, in the CSPN reactor. Evidently, NO_2^--N accumulation and phenol removal could be rapidly carried out in the CSPN reactor. The ratio of NO_2^--N to NH_4^+-N reached around 1.05. Between days 86 and 125, the concentrations of NH_4^+-N and NO_2^--N were about 4.7 and 6.4 mg L^{-1} in the effluent of anammox reactor, and the corresponding removal efficiency of total nitrogen (TN) reached about 82.4%. Thus, the CSPN/A process was successfully started within 15 days and operated stably for more than 100 days with 100 mg phenol L^{-1}. This time was shorter than the start-up period of previous studies such as 23 days (Liang et al. 2011), 36 days (Wyffels et al. 2004), and 67 days (Huang et al. 2016).

At the end of phase I, the activity of AOB only decreased from 133.60 ± 4.53 to 122.24 ± 2.45 mg N g^{-1} VSS day^{-1}, and the SAA of sludge slightly decreased from 73.20 ± 1.04 to 57.37 ± 2.20 mg N g^{-1} VSS day^{-1}. Furthermore, the NOB activity was completely suppressed in the CSPN reactor. Although the repression of NOB could be achieved by limiting the DO (Wu et al. 2016), the control level was high. In the CSPN reactor, the oxygen diffusion was easier to control due to high concentration of sludge and complete mixing. Because organic compounds promoted the heterotrophic nitrifying bacteria which competed with NOB for nitrite (Li et al. 2016), the proliferation of heterotrophic nitrifying bacteria was limited by the lower COD/N ratio (Wang et al. 2016). Consequently, the CSPN reactor could efficiently remove phenol and suppress the proliferation of heterotrophic nitrifying bacteria in the anammox reactor, providing a high-quality effluent for feeding the anammox bacteria. The heterotrophic nitrifying bacteria would compete with anammox bacteria for nitrite (Chen et al. 2016; Qin et al. 2017), and the membrane filtration of CSPN reactor not only avoided the loss of AOB but also

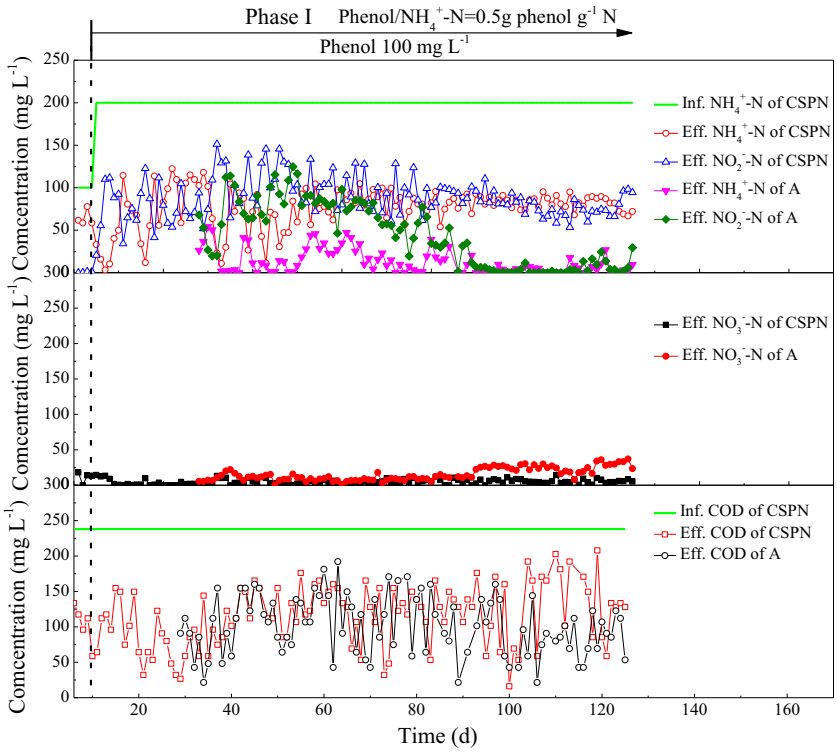

Fig. 3 Variations of NH_4^+-N, NO_2^--N, NO_3^--N, and COD concentration in the CSPN/A process at phase I (CSPN, completely separated partial nitritation stage; A, anammox stage; Inf., influent; Eff., effluent)

minimized the populations' competition in the anammox reactor.

Shock of phenol concentration (phase II) and the recovery performance (phase III) of the CSPN/A process

Variations of NH_4^+-N, NO_2^--N, NO_3^--N, and COD concentrations in the CSPN/A process at phases II and III are shown in Fig. 4. When the CSPN reactor was subjected to a shock concentration of 300 mg phenol L^{-1} at phase II, the phenol/NH_4^+-N ratio was increased from 0.5 (phase I) to 1.5 g phenol g^{-1} N (phase II) in the CSPN reactor. The effluent concentration of NH_4^+-N rapidly increased to about 166.5 mg L^{-1} and almost no NO_2^--N was detected. Consequently, the concentration of NH_4^+-N increased to about 169.60 ± 11.91 mg L^{-1} in the effluent of anammox reactor from days 153 to 161. The total nitrogen removal was completely lost in the anammox reactor. Interestingly, the activities of AOB and NOB, and the SAA of sludge were completely suppressed, but the SUR of sludge was significantly increased from 193.02 ± 1.75 (phase I) to 436.71 ± 10.73 mg phenol g^{-1} VSS day^{-1} (phase II) (as shown in Table 1). The shock of phenol concentration of 300 mg L^{-1} did not cause an accumulation of phenol in the effluent of CSPN reactor. Pereira et al. (2014) indicated that the depletion of phenol (300 mg L^{-1}) would increase the growth of denitrifying bacteria which could degrade aromatic compounds. Xu et al. (2016) observed that the relative abundance of AOB and denitrifying bacteria denoted opposite variations when phenol/NH_4^+-N ratio increased from 0.27 to 1.35, ranging from 30.1 to 17.5% and 7.6 to 18.2%, respectively. Therefore, the growth of heterotrophic bacteria would compete with AOB for oxygen at low DO concentration (Giustinianovich et al. 2016; Liang et al. 2014; Zhang et al. 2012). Furthermore, the inhibition of partial nitritation resulted in the long-term lack of nitrite in the anammox reactor. The populations' competition for a nitrite among three anammox bacteria was also found in the nitrite-limited bioreactor, contributing to the decrease of three species (Zhang et al. 2017). In the starvation experiment, the death and decay rates of anammox bacteria were 0.011 and 0.015 day^{-1}, respectively, and the death of anammox bacteria was the main cause of decreased activity under starvation conditions (Wang et al. 2018). Pereira et al. (2014) found that nitrogen removal efficiency of anammox was still around 90% at 200 mg L^{-1} phenol with phenol/NH_4^+-N of approximately 2. Yang et al. (2013) reported that 50 mg L^{-1} phenol with phenol/NH_4^+-N of 0.19 caused 89% inhibition on nitrogen removal in anammox reactor. Therefore, the long-term absence of nitrite was the main reason for the suppression of anammox bacteria. The performance failure of CSPN process could not be attributed to the toxicity of phenol, but the shift of microbial community structure was caused by the increase of phenol/NH_4^+-N ratio in the CSPN reactor.

Subsequently, the influent concentration of NH_4^+-N in the CSPN reactor was increased to 600 mg L^{-1} with the phenol/NH_4^+-N ratio decreasing to 0.5 g phenol g^{-1} N at phase III.

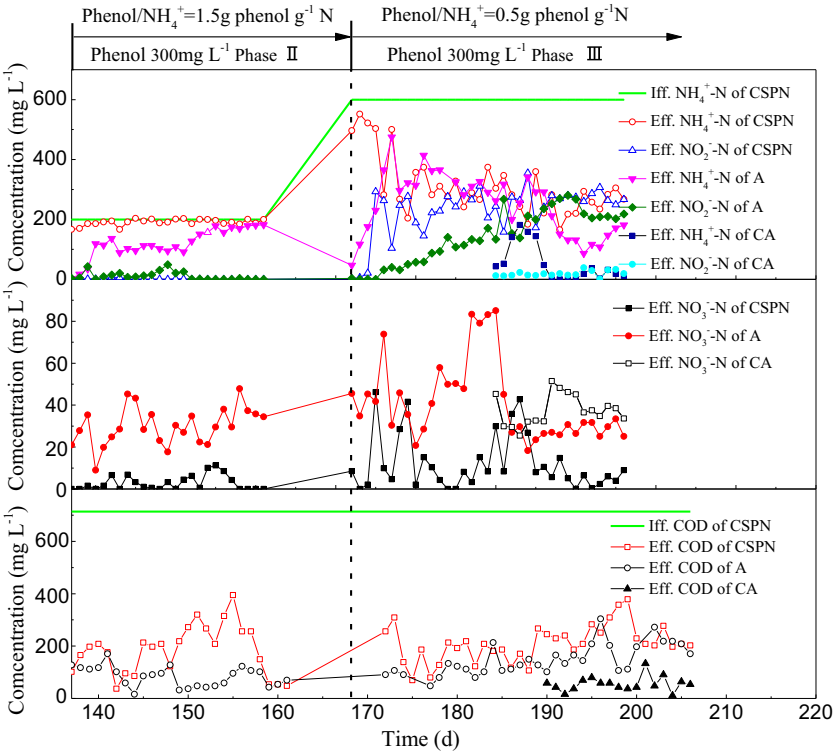

Fig. 4 Variations of NH_4^+-N, NO_2^--N, NO_3^--N, and COD concentrations in the CSPN/A process at phases II and III (CSPN, partial nitritation reactor; A, anammox reactor; CA, control anammox reactor; Inf., influent; Eff., effluent)

Table 1 The activities of AOB, NOB, and SUR of sludge in the CSPN reactor and SAA of sludge in the anammox reactor

Reactor	Activity*	Seed	Phase I	Phase II	Phase III
CSPN	AOB	133.60 ± 4.53	122.24 ± 2.45	0	144.36 ± 3.67
	NOB	53.71 ± 1.93	0	0	0
	SUR	202.32 ± 1.84	193.02 ± 1.75	436.71 ± 10.73	383.41 ± 9.02
Anammox	SAA	73.20 ± 1.04	57.37 ± 2.20	0	0
Control		130.10 ± 2.61	–	–	75.42 ± 5.07

*AOB activity, mg NH_4^+-N g^{-1} VSS day^{-1}; NOB activity, mg NO_2^--N g^{-1} VSS day^{-1}; SUR, mg phenol g^{-1} VSS day^{-1}; SAA, mg NH_4^+-N g^{-1} VSS day^{-1}

The activated sludge in a SBR reactor recovered the nitrogen removal efficiency in 52 days after stopping the addition of 30 mg L^{-1} of 2,4-dichlorophenol (Lim et al. 2012). Toh and Ashbolt (2002) found that nearly 30 days were required for recovery of partial nitritation when dosing of 250 mg L^{-1} phenol. The inhibition of 715 mg L^{-1} phenol shock needed 3 weeks to be overcome (Kim et al. 2011). Since day 191, the effluent concentrations of NO_2^--N and NH_4^+-N was about 264.6 and 254.4 mg L^{-1} at the mass ratio of NO_2^--N to NH_4^+-N of 1.05. At the phenol concentration of 300 mg L^{-1}, the CSPN reactor only spent 19 days to reestablish a stable performance. The activity of AOB was 144.36 ± 3.67 mg NH_4^+-N g^{-1} VSS day^{-1} at the end of phase III, which was higher than that of phase I. The shock of phenol concentration caused the inferiority of AOB to the oxygen utilization while the toxicity of phenol on the AOB. Therefore, the high concentration of phenol could be rapidly degraded by the efficient phenol degraders in the CSPN reactor, but the control of phenol/NH_4^+-N ratio played an important role for the recovery of AOB activity.

For the anammox reactor, although the HRT was extended to 34.6 h at phase III, the anammox bacteria were still irreversibly suppressed. In the control anammox reactor, the total nitrogen removal efficiency was more than 80% with the effluent NH_4^+-N, NO_2^--N, and NO_3^--N concentrations of about 19.60, 25.33, and 36.74 mg L^{-1}, respectively. The SAA of sludge in the control anammox reactor was around 75.42 mg NH_4^+-N g^{-1} VSS day^{-1} at the end of experiment. There was an increasing consensus that the anammox activity was severely inhibited by high concentration of nitrite (Bettazzi et al. 2010; Jaroszynski et al. 2011). Egli et al. (2001) pointed out that the anammox bacteria would be completely deactivated when the concentration of nitrite was more than 185 mg L^{-1}. Jetten et al. (1998) observed a complete inhibition of the anammox process when the concentration of nitrite was higher than 280 mg L^{-1}. At phase III, the influent concentration of nitrite was around 194.23 mg L^{-1} in the anammox reactor which could operate stably. The results indicated that the CSPN/A process was completely feasible to treat the wastewater containing 300 mg L^{-1} of phenol and 600 mg L^{-1} of NH_4^+-N.

Analysis of microbial community structure

The microbial community structure in the CSPN reactor at genus level is displayed in Fig. 5. The results showed a strong shift of microbial community structure among the three phases. In phase I, the relative abundances of dominant genera were *Pseudofulvimonas* (14.66%), *Gemmatimonas* (14.57%), *Nitrosomonas* (9.99%), *Aquimonas* (6.14%), *Zoogloea* (3.77%), *Thauera* (3.23%), and *Truepera* (2.69%). Among of them, the genus *Pseudofulvimonas* existed in the treatment of ammonium and high organic containing wastewater (El-Fadel et al. 2018). *Gemmatimonas* genus was able to metabolize a restricted range of substrates, such as benzoate, methoxy, and hydroxybenzoate (Braga et al. 2015). *Nitrosomonas* genus belonged to AOB which could oxidize ammonium (Liu et al. 2017a). *Zoogloea* genus was widely reported as phenol degrader under anaerobic conditions (Braga et al. 2015). *Thauera* genus was regarded as denitrifying bacteria, which could degrade nitrite and phenol (Garrity et al. 2005). As one of the most abundant genera, *Aquimonas* was found in a nitritation air-lift reactor under long-term HRT (Ali et al. 2016). *Truepera* genus exhibited positive relationships with degradation of aromatics (Zhang et al. 2015). Evidently, the dominant bacteria were phenol degrading and ammonium oxidizing bacteria, indicating that the microbial community structure was responsible for degrading phenol and oxidizing ammonium at the phenol/NH_4^+ ratio of 0.5.

After the shock of 300 mg phenol L^{-1} at phase II, the relative abundances of *Thermomonas*, *Thauera*, *Arenimonas*, and *Brevundimonas* increased to 19.50, 13.88, 11.74, and 9.73%, respectively. On the contrary, the relative abundance percentage of *Gemmatimonas* and *Nitrosomonas* decreased to 2.1 and 0.16%. *Thauera* was reported as denitrifying bacteria and phenol degrader (Garrity et al. 2005; Shinoda et al. 2004); however, there was still no denitrification due to complete inhibition of ammonium oxidation. Moreover, the abundance of *Thauera* increased with the higher loading of phenol, which indicated its strong tolerance to phenol toxicity. *Thermomonas* and *Arenimonas*, affiliated to denitrifying bacteria (Jia et al. 2016; Remmas et al. 2016), and *Brevundimonas*, which could

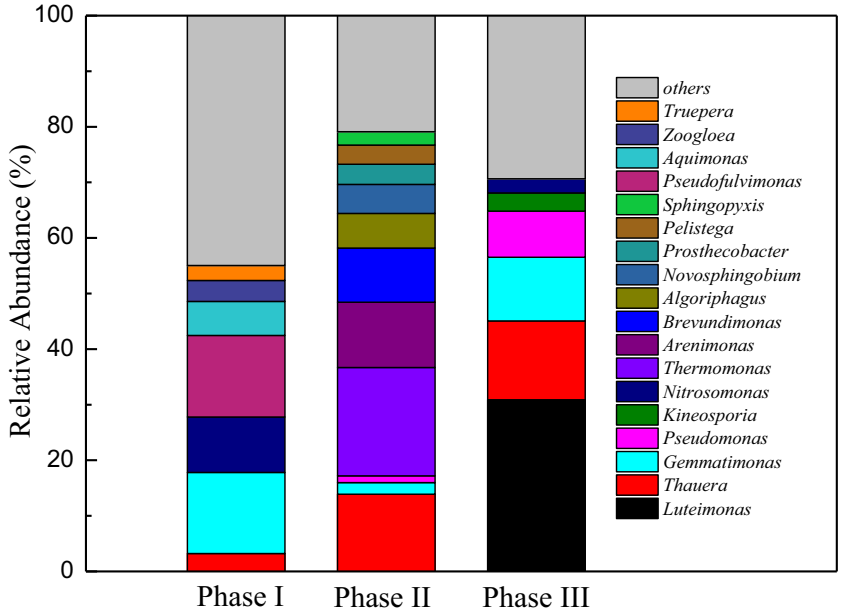

Fig. 5 Analysis of the microbial community structure in the CSPN reactor at genus level. "Others" represents all classified taxa that were below 2% in all samples

degrade aromatic compounds, sorely appeared at phase II. There was lack of nitrite and nitrate for the heterotrophic denitrifying bacteria, thus the denitrifying function was suppressed. *Thermomonas*, *Arenimonas*, and *Brevundimonas* were mainly responsible for organic degradation when the concentration of phenol increased to 300 mg L^{-1}. The reduction of the relative abundance of *Gemmatimonas* could be attributed to the increase of heterotrophic bacteria which could compete for substrate uptake. The decrease of the relative abundance of *Nitrosomonas* was related to its poor tolerance to phenol toxicity (Lauchnor and Semprini 2013).

When the phenol/NH_4^+-N ratio was decreased to 0.5 g phenol g^{-1} N at phase III, the relative abundance of *Nitrosomonas* was recovered to 2.55% in the CSPN reactor. *Luteimona* (30.89%), *Thauera* (14.17%), *Gemmatimona*s (11.46%), and *Pseudomonas* (8.31%) became the dominant phenol degraders at phase III. Emergent heterotrophic denitrifying bacteria, such as *Thermomonas* (0.31%), were suppressed when the partial nitritation was reestablished. These findings were in accordance with the aforementioned results obtained from the changes of AOB, NOB activities and SUR for phenol. Thereby, the efficient phenol degraders and phenol/NH_4^+-N ratio in the CSPN reactor played a key role to maintain the performance robustness of the anammox reactor. Moreover, the recovery of AOB and suppression of NOB could be carried out by controlling phenol/NH_4^+-N ratio at 0.5 with 300 mg phenol L^{-1} and 600 mg NH_4^+-N L^{-1}. Likewise, *Thermomonas*, dominating at phase II for denitrification, was severely suppressed, but the relative abundance of *Thauera*, *Gemmatimona*s, and *Pseudomonas* increased with the increase of phenol loading rate, which ensured the fast degradation rate of phenol. Denitrifying bacteria, such as *Thauera* and *Pseudomonas*, also appeared in the anammox reactor after the addition of phenol which could degrade phenol and alleviate its toxicity (Beristain-Cardoso et al. 2009; Pereira et al. 2014). Although the inhibition of anammox by phenol was reversible at a concentration of 50 mg phenol L^{-1} (Yang et al. 2013), the irreversible inhibition of SAA was observed when phenol was completely eliminated. As demonstrated in the present study, the potential impact of nitrite on the anammox reactor performance was remarkable. Although nitrite was the substrate of anammox bacteria, nitrite concentrations above a certain range would clearly have an inhibitory effect on anammox bacteria (Jin et al. 2012). Isaka et al. (2007) found that anammox was inhibited when nitrite concentration was more than 280 mg L^{-1}. Moreover, 50% inhibition of anammox activity was observed when the nitrite concentration increased to 400 mg L^{-1} (Lotti et al. 2012). As a result, the CSPN reactor could make the partial nitritation operated possible at 300 mg L^{-1} phenol, and the control of phenol/NH_4^+-N ratio was an important parameter to maintain the performance robustness of CSPN/A process treating phenol and ammonium containing wastewater.

Conclusion

The CSPN/A process could efficiently remove total nitrogen from the synthetic high-strength wastewater containing 300 mg L^{-1} of phenol and 600 mg L^{-1} of NH_4^+-N. Although the tolerance of anammox bacteria to phenol was higher than the AOB and NOB, the inhibition of AOB at phenol/NH_4^+-N ratio of 1.5 g phenol g^{-1} N caused a long-term lack of nitrite in the anammox reactor that was responsible for the suppression of anammox bacteria. Therefore, the high phenol concentration was not the limiting factor for the

anammox process, but the control of phenol/NH_4^+-N ratio was necessary to make the operation of the CSPN/A system possible at a phenol concentration of 300 mg L^{-1}.

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