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RESEARCH ARTICLE

Methanation and chemolitrophic nitrogen removal by an anaerobic membrane bioreactor coupled partial nitrification and Anammox

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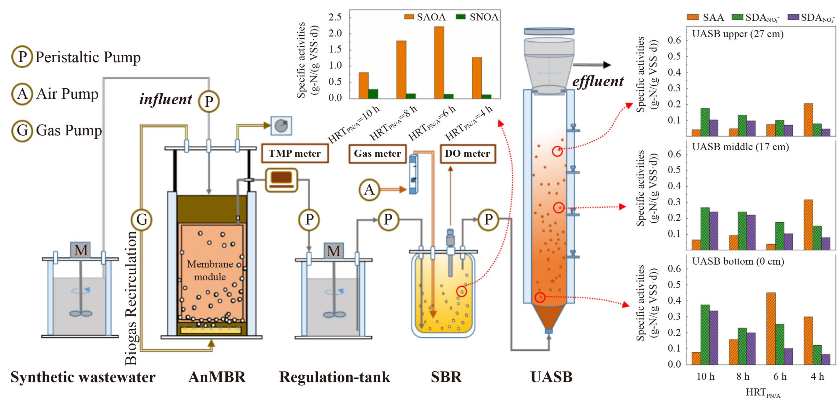
HIGHLIGHTS

- Efficient carbon methanation and nitrogen removal was achieved in AnMBR-PN/A system.
- AOB outcompeted NOB in PN section by limiting aeration and shortening SRT.
- The moderate residual organic matter of PN section triggered PD in anammox unit.
- AnAOB located at the bottom of UASB played an important role in nitrogen removal.

GRAPHIC ABSTRACT



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ABSTRACT

An AnMBR-PN/A system was developed for mainstream sewage treatment. To verify the efficient methanation and subsequent chemolitrophic nitrogen removal, a long-term experiment and analysis of microbial activity were carried out. AnMBR performance was less affected by the change of hydraulic retention time (HRT), which could provide a stable influent for subsequent PN/A units. The COD removal efficiency of AnMBR was > 93% during the experiment, 85.5% of COD could be recovered in form of CH_4. With the HRT of PN/A being shortened from 10 to 6 h, nitrogen removal efficiency (NRE) of PN/A increased from 60.5% to 80.4%, but decreased to 68.8% when the $HRT_{PN/A}$ further decreased to 4 h. Microbial analysis revealed that the highest specific ammonia oxidation activity (SAOA) and the ratio of SAOA to specific nitrate oxidation activity (SNOA) provide stable NO_2^--N/NH_4^+-N for anammox, and anammox bacteria (mainly identified as *Candidatus Brocadia*) enriched at the bottom of Anammox-UASB might play an important role in nitrogen removal. In addition, the decrease of COD in Anammox-UASB indicated partial denitrification occurred, which jointly promoted nitrogen removal with anammox.

1 Introduction

Anaerobic digestion (AD) is considered a promising energy-saving technology for wastewater treatment, during which organics could be converted to CH_4, thus decreasing CO_2 emissions. The AD effluent with low COD concentration and COD/N ratio can not achieve subsequent nitrogen removal by traditional nitrification/denitrification due to low carbon sources, thus an

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autotrophic biochemical process—partial nitrification/Anammox (PN/A)—is attractive because it has less aeration demand and excess sludge production and does not need an external carbon source. The effluent of AD matches PN/A needs to make this integration possible.

AD coupled with PN/A has been proposed to treat sewage and incinerate leachate (Gu et al., 2018; Sheng et al., 2020; Wang et al., 2022). Compared with up-flow anaerobic sludge blanket reactor (UASB) and anaerobic fixed-bed reactor (AFBR), an anaerobic membrane bioreactor (AnMBR) is more efficient for COD removal and CH_4 yield due to biomass retention capacity (Chen et al., 2017; Cogert et al., 2019; Vinardell et al., 2021). In addition, the low suspended solid (SS) concentration in AnMBR effluent is beneficial for the subsequent PN/A unit. Although attention has been paid to the performance of PN/A for treating AD effluent (Chen et al., 2020, Wang et al., 2020), information is limited on AnMBR coupled with PN/A (AnMBR-PN/A) for mainstream sewage treatment.

As an integrating process, the performance of each unit determined the containment removal efficiency of the whole system. AnMBR has been successfully used to treat municipal wastewater, and average COD removal can be > 87% even on a semi-industrial scale (Robles et al., 2022). The excessive nitrate in PN/A effluent results from the over-competition between nitrite-oxidizing bacteria (NOB) and ammonia-oxidizing bacteria (AOB) in PN and the anammox reaction seems to be an obstacle to promoting the contaminant removal efficiency in AnMBR-PN/A for mainstream sewage treatment (Kumar and Lin., 2010). Nevertheless, the residual organics in AnMBR effluent could probably trigger denitrification in PN/A, which is beneficial for improving nitrogen removal efficiency (NRE) (Hou et al., 2022). It should be noted that the maximum growth rate of denitrifying bacteria (DNB) is 7.2 d^{-1}, which is larger than the 0.0984 d^{-1} of anammox bacteria (Strous et al., 1998; Meng et al., 2020), indicating DNB are easy to over-proliferate and outcompete anammox bacteria (AnAOB) for NO_2^--N, consequently decreasing the NRE when the carbon source is sufficient (Tang et al., 2010; Du et al., 2014; Du et al., 2019). It is expected that organics should be only sufficient for converting NO_3^--N to NO_2^--N, which is called partial denitrification (PD), thus avoiding the competition of DNB for AnAOB (Kartal et al., 2008; Zhang et al., 2020). As one of the crucial parameters to consider in maximizing AnMBR-PN/A performance, hydraulic retention time (HRT) affects carbon removal and nitrogen conversion efficiency of AnMBR which mediates the interspecific cooperation/competition of functional microorganisms in the subsequent PN/A. It also has a direct influence on microbial properties of the PN and Anammox units (Ma et al., 2020; Lei et al., 2021). The microbial community is critical for determining system performance, therefore, elucidating the microbial mechanisms is necessary for optimizing AnMBR-PN/A performance for treating sewage.

In this study, an AnMBR-PN/A system was developed to verify the feasibility of efficient methanation and subsequent chemolitrophic nitrogen removal. With 16S rRNA high-throughput sequencing, specific microbial activity tests, and fluorescence in situ hybridization (FISH), we investigated the succession of the microbial community and the variation of microbial activities under different HRTs. We aimed to clarify the microbial mechanisms for the efficient performance of AnMBR-PN/A.

2 Materials and methods

2.1 The AnMBR-PN/A setup and operation

A lab-scale integrated process (Fig. S1) was developed to achieve concurrent carbon separation and nitrogen removal. A submerged AnMBR with a working volume of 6 L was used for carbon separation. A polyvinylidene fluoride (PVDF) micro-filtration membrane module (Chongqing Jichuang Technology, China) was used to achieve solid-liquid separation. The pore size of the membrane module was 0.2 μm and the total surface area was 0.1 m^2. The gas in the headspace was recycled by a diaphragm pump. A sequencing batch reactor (SBR) with a working volume of 1.1 L was used for the PN unit (PN-SBR). Each cycle of PN-SBR consisted of feeding, intermittent aeration (the dissolved oxygen (DO) concentration was 0.3–0.5 mg/L), settling, and discharging stages. A peristaltic pump (BT100J-1A, Shanghai Cancun Instrument Equipment Company, China) was used to transfer PN-SBR effluent to the bottom of an up-flow anaerobic sludge bed (UASB) reactor with a working volume of 2.2 L, which was used for Anammox (Anammox-UASB).

The temperature was maintained at 25.0 °C ± 1.0 °C using a water bath. The details of the AnMBR-PN/A are shown in Table 1. The HRTs varied during the experimental stage during the 217 days of operation of the system. The HRTs of the AnMBR (HRT_{AnMBR}) were set at 16, 12, 10, and 7 h on days 1–33, 34–89, 90–166, and 167–217, respectively. As the volume of each reactor was fixed, the corresponding HRTs of PN-SBR (HRT_{PN}) were 3.3, 2.7, 2, and 1.3 h, respectively, and the HRTs of Anammox-UASB ($HRT_{Anammox}$) were set at 6.7, 5.3, 4, and 2.7 h. As such, the sum of HRT_{PN} and $HRT_{Anammox}$ ($HRT_{PN/A}$) were 10, 8, 6, and 4 h, and the sum of HRT_{AnMBR} and $HRT_{PN/A}$ (HRT_{Total}) was 26, 20, 16, and 11 h. When the HRT_{AnMBR} of the carbon separation was shortened, the corresponding $HRT_{PN/A}$ of nitrogen removal also changed.

Table 1 Operating conditions of the AnMBR-PN/A

Time (d)	HRT_{AnMBR} (h)	HRT_{PN} (h)	$HRT_{Anammox}$ (h)	$HRT_{PN/A}$ (h)	HRT_{Total} (h)
1–33	16	3.3	6.7	10	26
34–89	12	2.7	5.3	8	20
90–166	10	2.0	4.0	6	16
167–217	7	1.3	2.7	4	11

2.2 Synthetic sewage and seed sludge

The influent of AnMBR was synthetic wastewater with COD and TN concentrations of 500.0 ± 45.0 and 50.0 ± 3.0 mg/L, respectively, and a total phosphorus concentration of 5.0 ± 1.0 mg/L, to simulate domestic wastewater, according to the detailed chemical composition described by Chen et al. (2017). The pH was not controlled and fluctuated between 7.54 and 7.96. The seeding sludge used for AnMBR was taken from a UASB used for anaerobic brewery wastewater digestion at the Hans Brewery (Xi'an, China). The initial mixed liquor volatile suspended solid (MLVSS) was approximately 8.0 g/L. The seed sludge used for PN-SBR and Anammox-UASB was collected from the anoxic tanks of the A/A/O at the sewage treatment plant (Xi'an, China). AnAOB had been successfully enriched in the anoxic tanks (Li et al., 2019). The initial MLVSS concentrations used in PN-SBR and Anammox-UASB were 1.52 g/L and 4.65 g/L, respectively.

2.3 Analytical methods

The ammonium, nitrite, nitrate, COD, and mixed liquor (volatile) suspended solids (MLSS/MLVSS) were measured according to the Standard Methods. The proportion of components in the biogas and the volume of dissolved methane in the effluent was determined by the method described in our previous study (Chen et al., 2017). The pH, temperature, and DO concentration were measured by a HACH HQ30d corresponding probe (LDO101, HACH Company, USA).

2.4 A batch test for specific microbial activity

During the start-up and steady operation of AnMBR-PN/A, AnMBR could be started quickly and maintained in a steadily running condition. However, the slow-growing AnAOB was a rate-limiting step in the PN/A system. Therefore, the specific microbial activity in AnMBR was not detected. A series of batch experiments were carried out to test specific ammonia oxidation activity (SAOA), specific nitrate oxidation activity (SNOA), specific anammox activity (SAA), and specific denitrification activity (including SDA_{NO_3} and SDA_{NO_2}, where the nitrogen sources were nitrate and nitrite, respectively) at the end of each stage. The substance concentration in different activity tests is shown in Table S1. The temperature was maintained at 25.0 ± 1.0 °C in all of the batch tests, which was the same as the reactor.

2.5 Fluorescence in-situ hybridization (FISH)

The flocs and granule samples taken from the PN-SBR/Anammox-UASB system on days 143 and 210 were analyzed by the fluorescence *in-situ* hybridization (FISH) method. The details of FISH and the sample observation methods were according to our previous study (Chen et al., 2017). In particular, the floc sludge was not sliced. The details of the probes are shown in Table S2.

2.6 Microbial community analysis

Four samples were collected on day 143 (stage Ⅲ) from the PN-SBR and Anammox-UASB at different heights to investigate the composition of the microbial community in the reactor. DNA for high-throughput sequencing was extracted using a PowerSoil DNA Isolation Kit (MO BIO, USA) according to the manufacturer's guidelines. The bacterial 16S rRNA gene was amplified with the primers 341F (5′-CCTACGGGNGGCWGCAG-3′) and 805R (5′-GACTACHVGGGTATCTAATCC-3′) for the V3 and V4 regions. The DNA library was constructed and run on the Miseq Illumina platform. Low-quality sequences were removed and high-quality sequences were processed to generate operational taxonomic units (OTUs) by ImageJ at a 97% sequence similarity threshold.

3 Results and discussion

3.1 The conversion and removal of pollutants in the AnMBR-PN/A

3.1.1 COD conversion in the AnMBR

As the carbon separation unit, AnMBR could achieve effective COD removal and CH_4 yield as shown in Figs. 1(a) and 1(b) and Table 2. The average COD concentration of the AnMBR effluent was consistently less than 30.0 mg/L and the COD removal efficiency in AnMBR was > 93% during the experiment. The membrane of AnMBR was cleaned every two months to maintain the transmembrane pressure (TMP) below 20 MPa. With the variation of HRT, the proportion of CH_4 in the biogas was maintained high at 87.3% ± 2.0%, while the

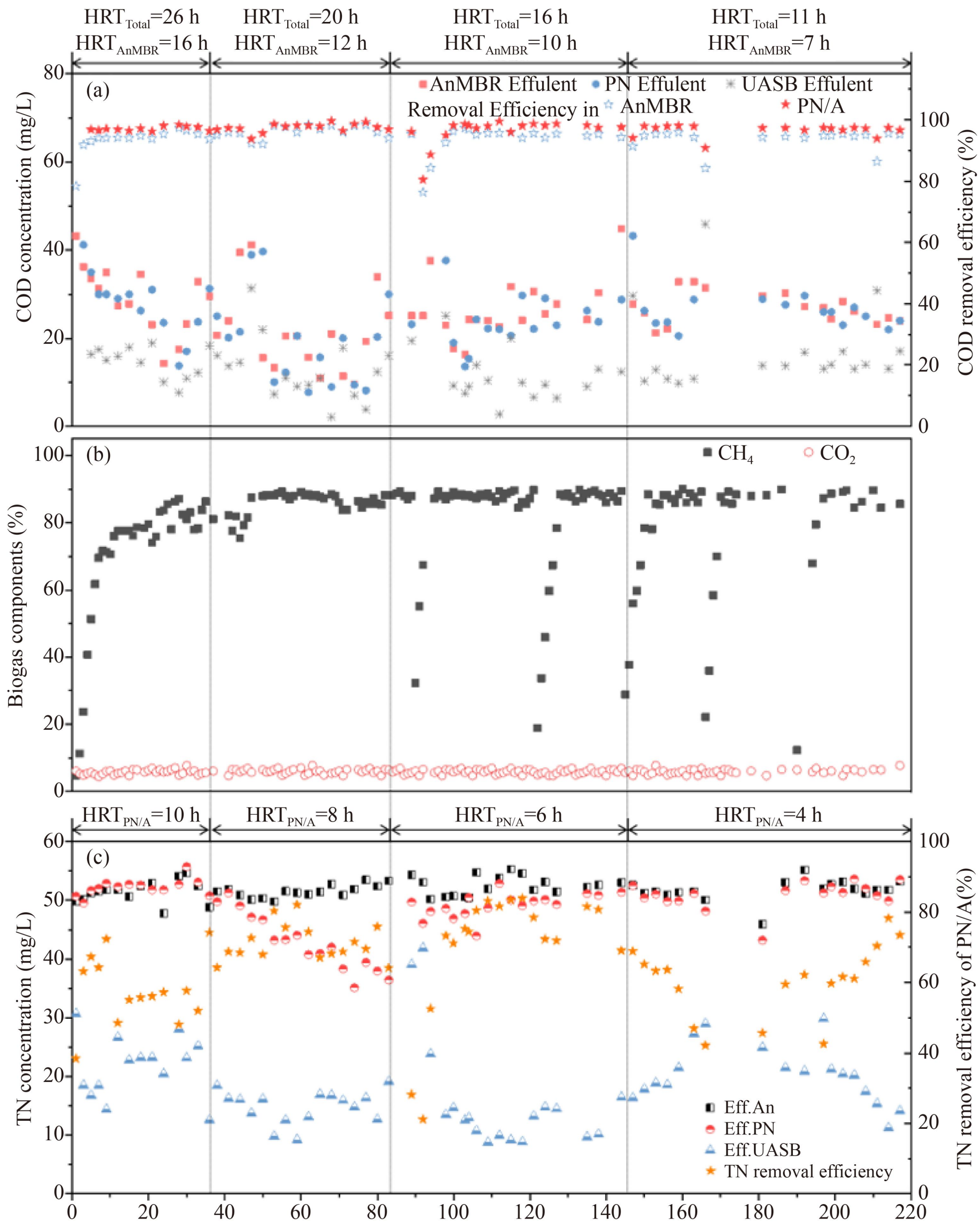

Fig. 1 (a) COD removal performance in the AnMBR-PN/A; (b) the proportion of CH_4 and CO_2 in the biogas; (c) total nitrogen removal performance in PN/A.

Table 2 Conversion and removal effects of COD and nitrogen under different HRTs

HRT_{Total} (h)	HRT_{AnMBR} (h)	$HRT_{PN/A}$ (h)	Proportion of CH_4 in biogas of AnMBR (%)	COD removal efficiency of AnMBR (%)	COD removal efficiency of AnMBR-PN/A (%)	NRE of AnMBR-PN/A (%)
26	16	10	84.7±3.5	93.2±4.5	97.1±0.6	60.6±4.2
20	12	8	86.2±2.1	95.9±1.9	97.3±1.3	74.9±4.0
16	10	6	85.8±1.6	93.3±4.7	96.2±4.1	80.4±2.9
11	7	4	86.0±1.9	94.2±2.4	96.8±0.9	68.8±3.1

Notes: the removal efficiency of COD and nitrogen was calculated using the data from the stable performance.

proportion of CO_2 was kept low at 4.1% ± 2.0%, after stabilizing from start-up. The COD balance (Fig. S1) showed that 85.5% of COD converted to CH_4, which was determined by the biogas composition and production rate. The soluble CH_4 in the AnMBR effluent was 5.7% of total COD and the biomass of microbial growth accounted for 5.4%. The CH_4 yield reached 0.31 L-CH_4/g $COD_{removed}$, approaching the theoretical value of 0.35 L/g

COD. The COD removal efficiency and CH_4 yield of AnMBR were significantly higher than that of UASB and AFBR (Gu et al., 2018; Wang et al., 2022), indicating it was an optimal option for carbon separation. In addition, AnMBR performance was less affected by the change of HRTs, which could provide a stable influent for subsequent PN/A units.

The COD concentration of the PN-SBR effluent was always close to that of its influent, that is, the degradation capacity of PN-SBR for COD of AnMBR effluent was limited. However, the COD concentration of UASB effluent was significantly lower than that of the PN-SBR effluent, and it was <15.0 mg/L (almost 50% lower than that of AnMBR effluent). Total COD removal efficiencies of 98.3% in the AnMBR-PN/A system were observed, indicating that further removal of COD was achieved during the PN/A process. We speculate that denitrification probably occurred in Anammox-UASB using COD as a carbon source, which lowered the COD concentration.

3.1.2 Nitrogen removal in the PN/A

As illustrated in Fig. 1(c), with the $HRT_{PN/A}$ shortened from 10 to 6 h, the NRE of the system gradually increased. When the $HRT_{PN/A}$ was 6 h, the NRE reached 80.4% and the effluent TN concentration of PN/A was < 13 mg/L; however, when the $HRT_{PN/A}$ was shortened to 4 h, stable performance was disrupted. Compared with the AnMBR, the change of HRT had a significant impact on the PN/A unit. Therefore, maintaining an efficient and stable performance of the PN/A was crucial for the system.

For PN-SBR, the ratio of NO_2^--N to NH_4^+-N (NO_2^--N/NH_4^+-N) in the effluent was the key parameter (Choi et al., 2020), which needs to reach the theoretical value of 1.32 in the anammox reaction (Strous et al., 1999). The NO_2^--N produced was only 12.3 ± 2.1 mg/L, perhaps due to insufficient aeration at an $HRT_{PN/A}$ of 10 h, which resulted in a NO_2^--N/NH_4^+-N value <1.32 (Fig. S2). With the increase of aeration at $HRT_{PN/A}$ of 8 h, the concentration of NO_3^--N increased and NO_2^--N decreased, indicating NOB out-competed AOB. Therefore, the aeration mode was changed from 3 min on, 40 s off to 4 min on, 30 s off. The SRT of the PN unit was shortened from 30 to 7 days to control the proliferation of NOB. When the $HRT_{PN/A}$ was 6 h, NO_3^--N concentrations in the PN-SBR effluent were relatively stable and the average concentration was 5.04 mg/L. The ratio of NO_2^--N/NH_4^+-N in the PN-SBR effluent was 0.66 ± 0.24 at an $HRT_{PN/A}$ of 6 h, which approached the 1.32.

The nitrogen removal efficiency of Anammox-UASB is shown in Fig. 2 Compared with the $HRT_{PN/A}$ 10 and 8 h, the nitrogen in the effluent at an $HRT_{PN/A}$ of 6 h remained at a relatively low level. But the NRE decreased when the $HRT_{PN/A}$ was changed to 4 h, and the NH_4^+-N and NO_2^--N co-existed in the UASB effluent. The ratio of ΔNO_2^--N/ΔNH_4^+-N in Anammox-UASB was <1.32 most of the

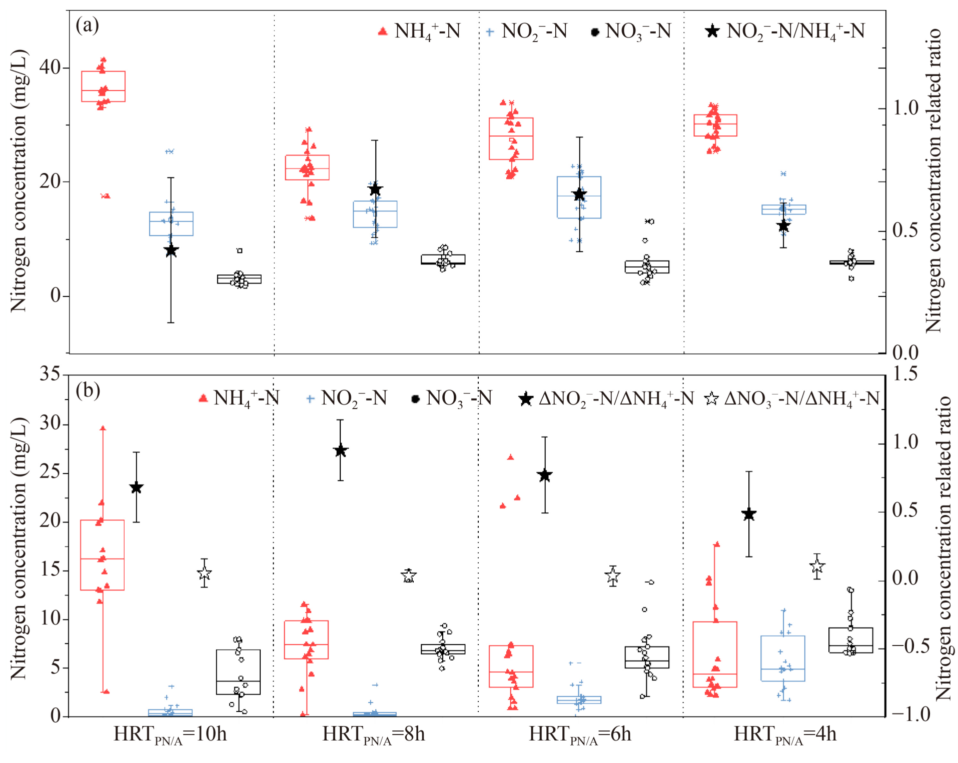

Fig. 2 (a) Nitrogen concentrations and the NO_2^--N/NH_4^+-N value of the PN-SBR effluent at various HRTs; (b) nitrogen concentrations, ΔNO_2^--N/ΔNH_4^+-N, and ΔNO_3^--N/ΔNH_4^+-N of the Anammox-UASB at various HRTs.

time, and the ratio of ΔNO_3^--N/ΔNH_4^+-N in Anammox-UASB was always less than the stoichiometry of Anammox at 0.26 (Fig. S3). Combined with the significant decrease of COD concentration in UASB, it can be speculated that the partial denitrification (PD) process (NO_3^--N→NO_2^--N) in the UASB consumed nitrate and COD (Cao et al., 2019).

3.2 Microbial activities associated with nitrogen conversion and removal

3.2.1 Microbial activities in the PN-SBR

To clarify the microbial mechanism of efficient nitrogen removal, the specific microbial activities of AOB and NOB in PN-SBR were tested on days 30, 80, 143, and 210 (Fig. 3(a)). It was clear that SAOA was larger than SNOA throughout the experiment. In particular, the SAOA-to-SNOA ratio (SAOA/SNOA) was up to 12:1 at an $HRT_{PN/A}$ of 6 h. The AOB in the PN-SBR was mainly *Nitrosomonas*, and the relative abundance was 0.17%; the NOB was mainly *Nitrospira* and the relative abundance was only 0.04% (Fig. 3(b)). It was also shown that AOB was enriched and NOB was inhibited. Several studies have shown that part of the NOB bacteria, such as some *Nitrospira,* adapt to a low DO environment (Cao et al., 2017; Zhang et al., 2019). Regulation of SRT could lead to changes in the microbial community in the PN-SBR because the minimum doubling time of AOB (7–8 h) was shorter than that of NOB (10–13 h) (Peng and Zhu, 2006). This could explain why the intermittent aeration and SRT control effectively inhibited the NOB activity. However, SAOA decreased to 1.27 g-N/g VSS/d due to high NLR (0.9 g-N/m^3/d) at an $HRT_{PN/A}$ 4 h, which should be the main reason for the change in nitrogen conversion of PN-SBR.

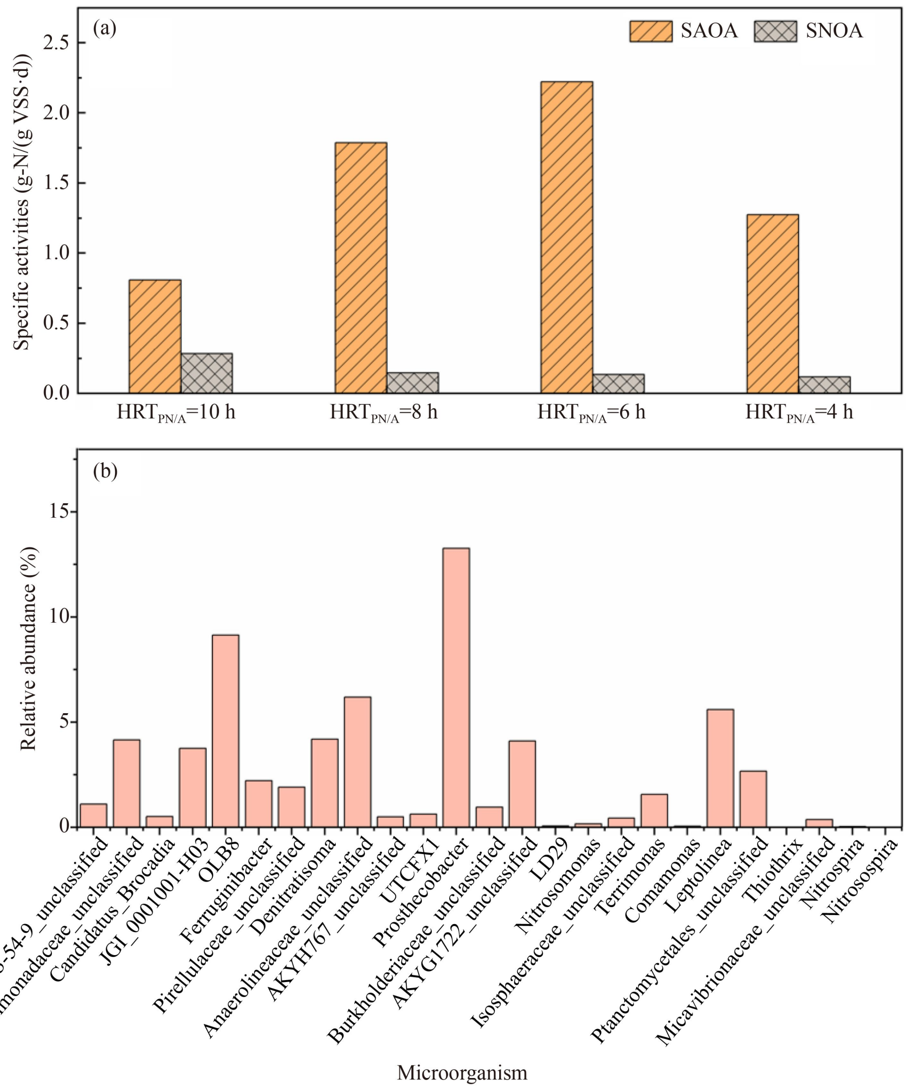

Fig. 3 (a) Specific microbial activity of AOB and NOB in the PN-SBR; (b) relative abundance of major microorganisms in the PN-SBR when $HRT_{PN/A}$ was 6 h.

3.2.2 Microbial activities in Anammox-UASB

The nitrogen was removed in Anammox-UASB, and the specific microbial activities related to nitrogen conversion in Anammox-UASB were tested at different heights on days 30, 80, 143, and 210 as shown in Fig. 4. The positions 0, 17, and 27 cm from the bottom of the reactor were marked as bottom, middle, and upper, respectively. The SAA, SDA_{NO_3}, and SDA_{NO_2} could be detected in Anammox-UASB, and their value was highest at the bottom, compared with middle and upper positions, which might be due to the sufficient substrate at the bottom of Anammox-UASB. Thus, the specific microbial activities significantly decreased along with the height of Anammox-UASB.

SAA increased significantly from 0.078 to 0.452 g-N/g-VSS/d at the bottom of Anammox-UASB with the $HRT_{PN/A}$ shortened from 10 to 6 h. There was no significant difference between the upper and middle heights. The relative abundance of AnAOB (mainly *Candidatus Brocadia*) at the bottom of Anammox-UASB reached a maximum of 21.01% (Fig. 5). However, when the $HRT_{PN/A}$ was shortened from 6 to 4 h, SAA increased at the upper and middle of the Anammox-UASB, but decreased at the bottom. The SAA in the middle of Anammox-UASB was 0.338 g-N/g-VSS/d which was higher than that at the bottom (0.301 g-N/g-VSS/d). The variation of SAA and the abundance of AnAOB at the bottom of Anammox-UASB increased with $HRT_{PN/A}$ shortened from 10 to 6 h, then decreased with $HRT_{PN/A}$ shortened from 6 to 4 h, which was positively correlated to the variation of NRE. This indicated the AnAOB probably played an important role in nitrogen removal. The increased nitrogen load likely supplied sufficient substrate for the growth of AnAOB at the bottom of Anammox-UASB. The higher SAA in the middle of Anammox-UASB under $HRT_{PN/A}$ of 4 h may result from the high hydraulic load and the gas production which could float anammox granules at the bottom of the UASB.

SDA_{NO_3} and SDA_{NO_2} were highest at $HRT_{PN/A}$ of 10 h, the main reason being that the seed sludge was taken from the anoxic tank of an A/A/O wastewater plant which had high DNB activity. Similar to SAA, the SDA_{NO_3} and SDA_{NO_2} at the bottom were highest, and then decreased with the concentration gradient of the substrate along with the height of Anammox-UASB. With the decrease of $HRT_{PN/A}$, the activity of DNB was gradually inhibited. But the SDA_{NO_3} was always greater than SDA_{NO_2}, indicating the DNB preferred to use nitrate rather than nitrite as the electron acceptor due to insufficient organic matter as reported in a previous study (Du et al., 2016; Wang et al., 2020).

Compared with reducing nitrite, the greater the activity of microbial reducing nitrate, the stronger the ability to accumulate nitrite through the PD reaction. Therefore, the PD potential of sludge can be characterized by the value of SDA_{NO_3}/SDA_{NO_2}. There was a significant linear correlation between SAA and SDA_{NO_3}/SDA_{NO_2}, indicating high SAA was highly correlated with strong PD potential. In this study, the organics were well separated by AnMBR, and the residual organics with low concentration contained in the effluent of AnMBR could trigger the PD reaction during which the NO_3^--N could be converted to NO_2^--N, avoiding competition between DNB and anammox. Therefore, under $HRT_{PN/A}$ of 6 h, when the SAA reached its maximum, the NRE of PN/A was significantly improved with PD.

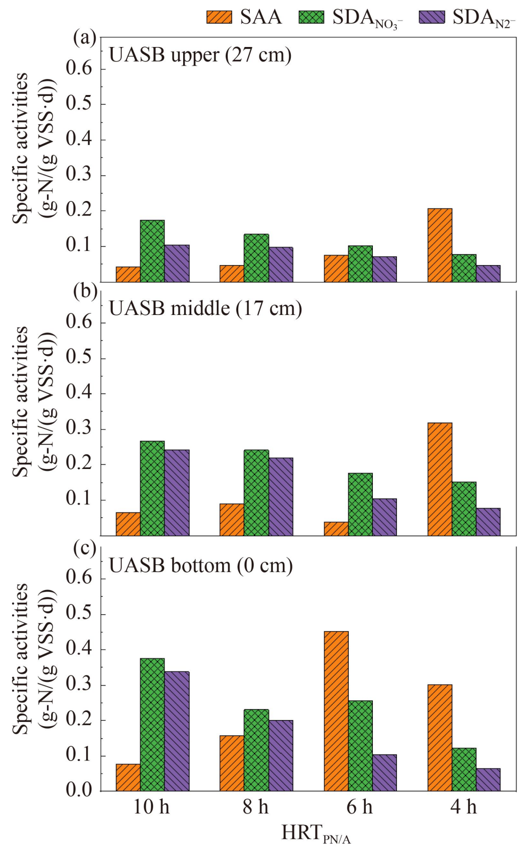

Fig. 4 Microbial activity in the Anammox-UASB: (a) UASB upper (27 cm), (b) UASB middle (17 cm), (c) UASB bottom (0 cm).

3.3 Spatial distribution of functional microorganisms

3.3.1 Spatial distribution of AOB in the PN-SBR

Fig. 6(a1–a4) shows the AOB and NOB distribution in the PN-SBR sludge on days 110 and 210 as determined by FISH combined with the use of a CLSM. The bright yellow signals in Fig. 6(a2) and (a4) resulted from the binding of EUBmix and AOBmix probes in one cell. The results indicated that the floc structure from the two samples on days 110 and 210 was similar, with microorganisms distributed throughout the flocs. The density of total bacteria in the flocs increased significantly, which showed that the biomass increased after 100 days of the experiment. The AOB accounted for a large proportion of whole flocs, but the signal of NOB

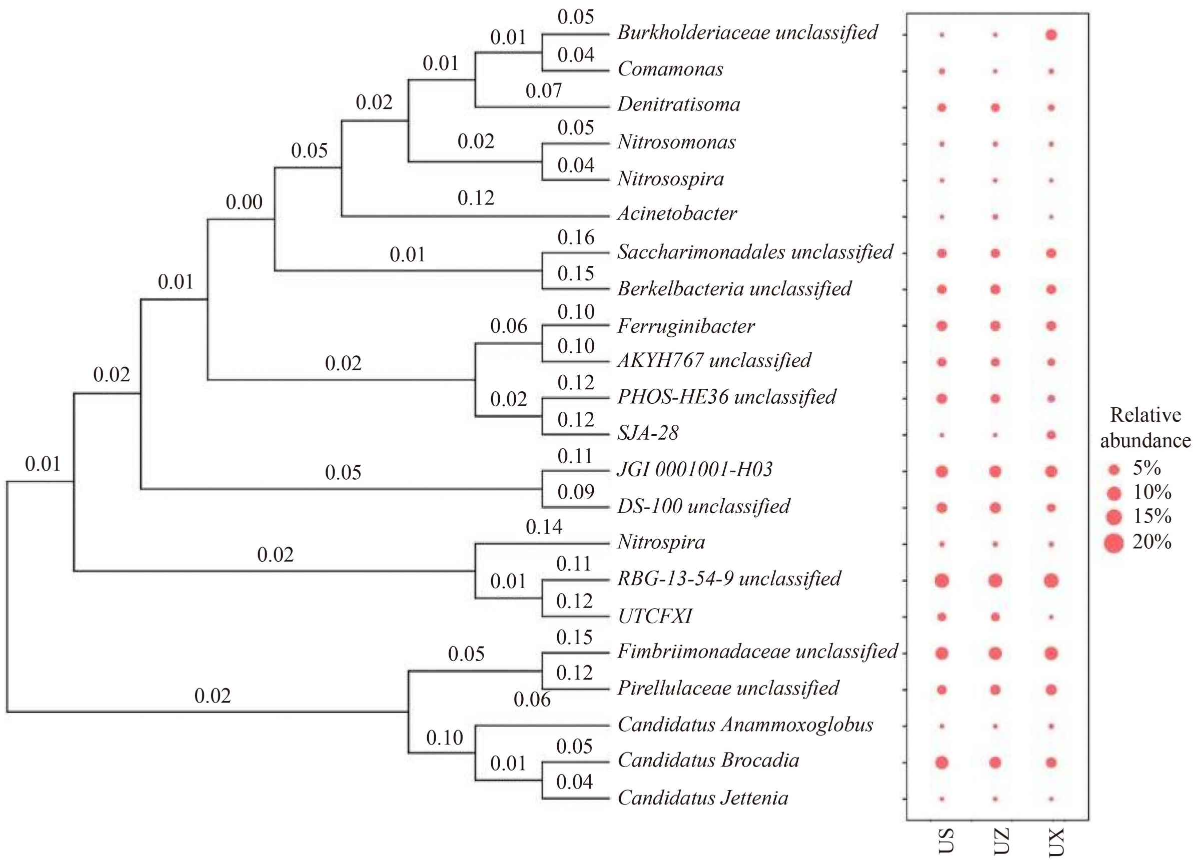

Fig. 5 Relative abundance of major microorganisms in the Anammox-UASB system when $HRT_{PN/A}$ was 6 h. Numbers on the branches of the phylogenetic tree indicate the branch distance. On the horizontal axis, US, UZ, and UX, respectively, indicate that the samples are taken from the upper, middle, and bottom of the UASB.

was weak. This phenomenon was consistent with the results of specific microbial activity of AOB and NOB discussed in 3.2.1, i.e., SAOA was significantly higher than SNOA.

3.3.2 Spatial distribution of Anammox in the Anammox-UASB

The results of specific activity tests showed that the activity of AnAOB was concentrated in the bottom of the Anammox-UASB, so FISH was mainly carried out using the sludge at the bottom. Anammox granules were obtained from the bottom of the UASB on day 110. Fig. 6(b1) shows the image of a mature granule and Fig. 6(b2) shows a single granule under the 40-fold lens of the confocal laser scanning microscope with the phone through the eyepiece. The image of a point inside the granular sludge and a point at the edge of the granular sludge is shown in Fig. 6(b3–b5) and Fig. 6(b6–b8), respectively. The AnAOB in the core of the granule accounted for an average of 95.2% ± 2.1% of the total bacteria, whereas the AnAOB at the edge accounted for 52.9% ± 5.3%. Inside the granule, the structures were not dense and AnAOB existed in clusters. At the edge of granular sludge, there were AnAOB coated with other microorganisms, which likely were AOB (Chen et al., 2019). This may be the main reason why the abundance of AnAOB in the outer layer is lower than that in the inner layer of the granule. AnAOB will be inhibited by long-term exposure to DO, so the inclusion of AOB outside the granule was more conducive to the growth of AnAOB, likely indicating a synergistic effect between AnAOB and AOB at the bottom of the Anammox-UASB (Hubaux et al., 2015).

As shown in Fig. S4, the proportion of sludge with particle size <0.2 mm decreased significantly with the HRT decreasing, indicating that fast granulation occurred during the long-term experiment. At the bottom of Anammox-UASB, the proportion of sludge with a particle size >0.9 mm decreased from 13.7% to 10.5% with the $HRT_{PN/A}$ shortening from 6 to 4 h, which probably resulted from the increase in the up-flow rate.

3.4 COD and nitrogen conversion path in the AnMBR-PN/A

Based on the performance of AnMBR-PN/A at $HRT_{PN/A}$ of 6 h, which was the optimal operational condition, the

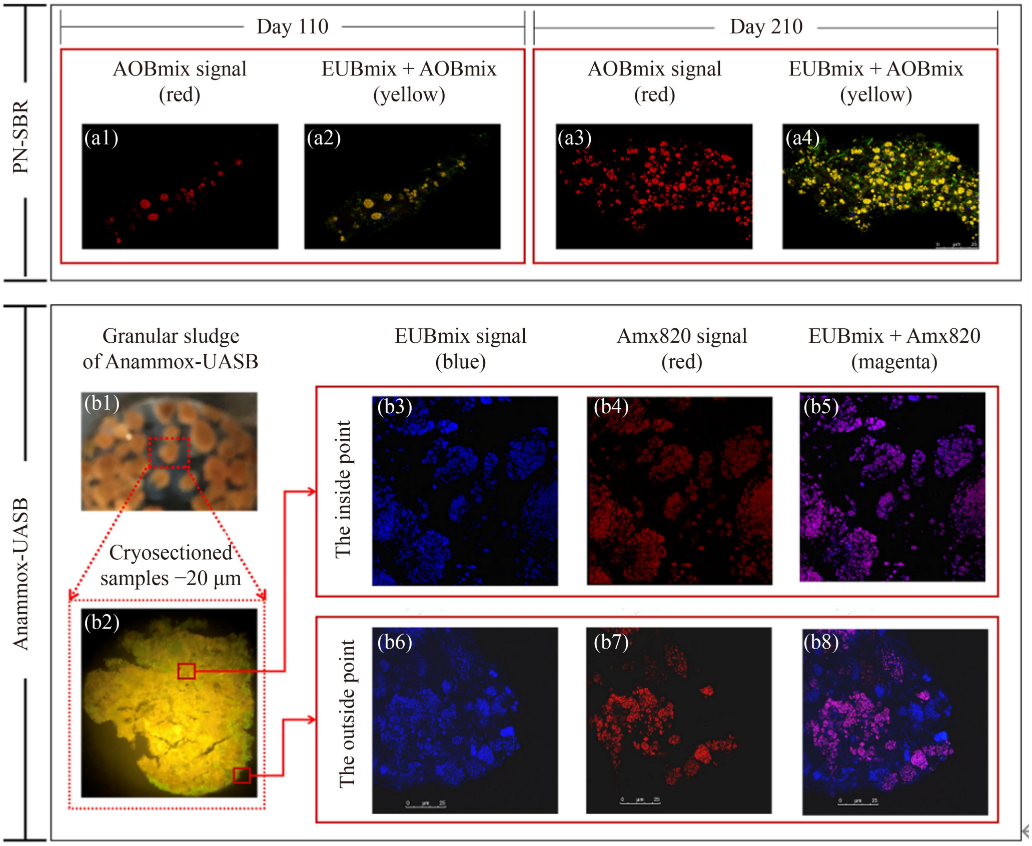

Fig. 6 Spatial distribution of functional microorganisms in the PN-SBR and Anammox-UASB.

COD and nitrogen conversion path are illustrated in Fig. 7. The COD of sewage was first separated in AnMBR through methanogenesis. Then, the NH_4^+-N contained in AnMBR effluent was converted to NO_2^--N in PN-SBR mainly by *Nitrosomonas*—which was outcompeted by NOB. Subsequently, COD and TN removal was achieved under the synergistic action of AnAOB (mainly *Candidatus_Brocadia*) and DNB (mainly *Denitratima*) in the Anammox-UASB. The NH_4^+-N and NO_2^--N in the PN-SBR effluent entered the interior of the granular sludge through the pores, and produced N_2 and nitrate accounting for 77.54% and 9.8% of TN, respectively, through Anammox. Since the mass transfer rate of COD is lower than that of ammonia, nitrite, and nitrate, *Denitratisoma* was mainly outside the granular sludge (Hubaux et al., 2015). COD reacted with nitrate in the outer granular sludge and produced nitrite accounting for 6.1% of TN, which once again became the matrix for anammox. Therefore, the layered structure of granular sludge in the Anammox-UASB protected AnAOB, and the PD reaction in the outer granular sludge supplemented the lack of nitrite-nitrogen, to improve the NRE (Li et al, 2022). Overall, the methanogenic and COD removal performance of the AnMBR not only realized energy recovery but also provided low COD/N influent for the PN/A system, which provided appropriate conditions for the stable operation of PN/A and the coupling of Anammox and PD.

Compared with the traditional aerobic sewage treatment process, the COD in the AnMBR-PN/A system was converted to CH_4 instead of being converted to CO_2. The residual COD in AnMBR effluent could be used as a carbon source to trigger PD which could couple with anammox to improve nitrogen removal without external carbon sources. Therefore, the AnMBR-PN/A provides considerable benefits for engineering applications. To achieve efficient methanation and chemolitrophic nitrogen removal of AnMBR-PN/A, the stable performance of the PN/A unit was crucial, because it was easier to be affected by operational condition changes. However, for mainstream municipal sewage, temperature should be the critical factor, as both the membrane fouling and the decrease of microbial activity (especially anammox bacteria) are aggravated by a temperature decrease (Guo et al., 2022). Further strategies for maintaining stable and efficient AnMBR-PN/A performance for mainstream sewage treatment are needed.

4 Conclusions

This study successfully integrated AnMBR and PN/A, and an excellent CH_4 conversion rate (85.5%) and NRE (80.4%) were achieved at an $HRT_{PN/A}$ of 6 h. AOB was

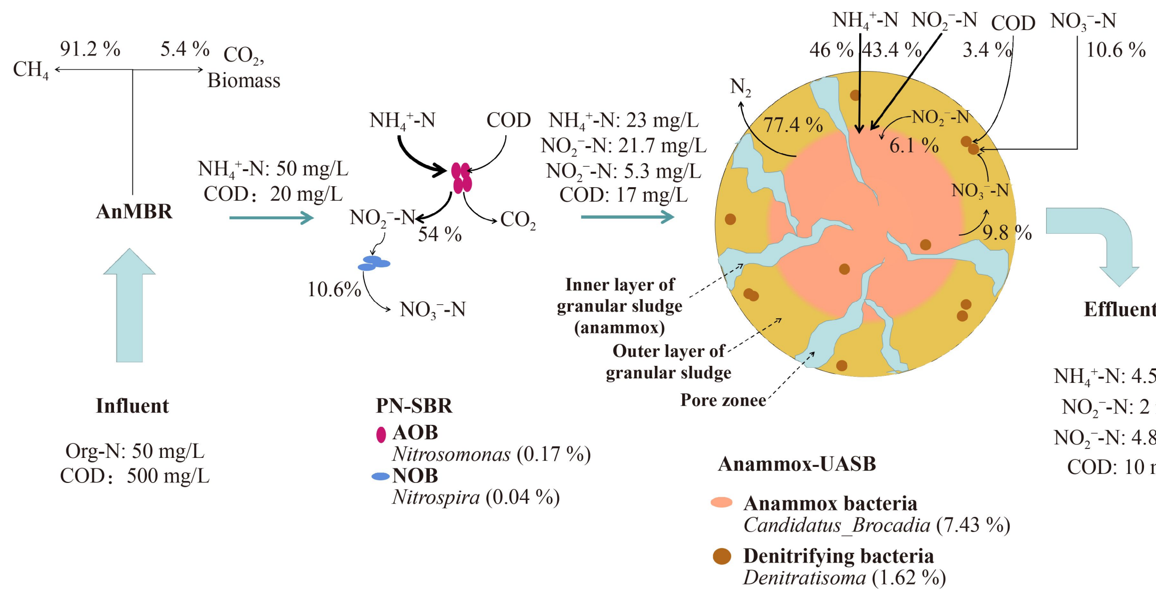

Fig. 7 COD and nitrogen conversion paths in the AnMBR-PN/A system.

enriched and NOB was effectively inhibited in the PN-SBR via the control of intermittent aeration and short SRT when the $HRT_{PN/A}$ was 6 h. The residual organic matter triggered effective PD in the Anammox section to provide NO_2^--N, so PD and Anammox jointly improved the NRE. The AOB was inhibited by residual organic matter, and sludge disturbance in the Anammox-UASB was destroyed when the influent load was too high ($HRT_{PN/A}$ of 4 h).

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