High performance nitrogen removal through integrating denitrifying anaerobic methane oxidation and Anammox: from enrichment to application

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ABSTRACT

Integrating denitrifying anaerobic methane oxidation (DAMO) with Anammox provides alternative solutions to simultaneously remove nitrogen and mitigate methane emission from wastewater treatment. However, the practical application of DAMO has been greatly limited by slow-growing DAMO microorganisms living on low-solubility gaseous methane. In this work, DAMO and Anammox co-cultures were fast enriched using high concentration of mixed sludges from various environments, and achieved nitrogen removal rate of 76.7 mg NH_4^+-$N L^{-1}$ d^{-1} and 87.9 mg NO_3^--$N L^{-1}$ d^{-1} on Day 178. Subsequently, nitrogen removal rate significantly decreased but recovered quickly through increasing methane flushing frequency, indicating methane availability could be the limiting factor of DAMO activity. Thus, this work developed a novel $\underline{M}$embrane $\underline{A}$erated $\underline{M}$embrane $\underline{B}$ioreactor (MAMBR), which equipped with gas permeable membrane for efficient methane delivery and ultrafiltration membrane for complete biomass retention. After inoculated with enriched sludge, nitrogen removal rates of MAMBR were significantly enhanced to 126.9 mg NH_4^+-$N L^{-1}$ d^{-1} and 158.8 mg NO_3^--$N L^{-1}$ d^{-1} by membrane aeration in batch test. Finally, the MAMBR was continuously fed with synthetic wastewater containing ammonium and nitrite to mimic the effluent from partial nitritation. When steady state with nitrogen loading rate of 2500 mg $N L^{-1}$ d^{-1} was reached, the MAMBR achieved total nitrogen removal of 2496.7 mg $N L^{-1}$ d^{-1}, with negligible nitrate in effluent (~6.5 mg NO_3^--$N L^{-1}$). 16S rRNA amplicon sequencing and fluorescence in situ hybridization revealed the microbial community dynamics during enrichment and application. The high performance of nitrogen removal (2.5 kg $N m^{-3}$ d^{-1}) within 200 days operation and excellent biomass retention capacity (8.67 kg VSS m^{-3}) makes the MAMBR promising for practical application of DAMO and Anammox in wastewater treatment.

1. Introduction

Denitrifying anaerobic methane oxidation (DAMO) is a recently discovered process through anaerobically oxidizing methane to provide electrons for denitrification (Ettwig et al., 2008; Raghoebarsing et al., 2006), which advances our understanding of the global carbon and nitrogen cycles. DAMO process is mediated by two distinct groups of microorganisms, DAMO bacteria and DAMO archaea (Haroon et al., 2013). DAMO bacteria oxidize methane using intracellular oxygen from dismutation of nitric oxide generated from nitrite (Ettwig et al., 2010), while DAMO archaea utilize methane in reverse methanogenesis to provide electrons to reduce nitrate to nitrite. DAMO microorganisms have been found in various environments, particularly in nitrogen contaminated ecosystems with limited oxygen (van Kessel et al., 2018), such as freshwater sediments (Martinez-Cruz et al., 2018; Shen et al., 2014), wetland (Chen et al., 2015; Xu et al., 2018; Yang et al., 2017), paddy soil (Ding et al., 2016; Vaksmaa et al., 2017; Wang et al., 2012; Zhou et al., 2014) and peatland (Andert et al., 2012; Zhu et al., 2012). Wastewater treatment plants (WWTP) is an important source of methane emission, which were estimated to account for approximately 5% of total global methane emissions (Czepiel et al., 1993; El-Fadel and Massoud, 2001). Methane generated onsite at WWTPs during energy

recovery from anaerobic digestion could be as an expected electron donor for denitrification. In addition, anaerobic ammonium oxidation (Anammox) are capable of autotrophic nitrogen removal through oxidation of ammonium to dinitrogen gas with nitrite as electron acceptor (Guo et al., 2018), which is an energy-efficient and cost-effective nitrogen removal process. However, Anammox could not remove nitrate in the wastewater, but converts part (20%) of the nitrite to nitrate, resulting in insufficient nitrogen removal. Consequently, the effluent from Anammox requires further polish before discharge (Regmi et al., 2016). Through converting nitrate produced by Anammox, DAMO enables Anammox to achieve complete nitrogen removal (Xie et al., 2017; Xie et al., 2018). Therefore, the discovery of DAMO provides promising opportunities to simultaneously remove nitrogen and mitigate methane emission from wastewater treatment.

The doubling time for DAMO microorganisms is as long as 2 weeks or more (Chen et al., 2014; He et al., 2013), which makes it difficult to obtain DAMO biomass even with long duration of the enrichment procedure. Ettwig et al. (2009) enriched DAMO bacteria from sediments of four ditches draining agricultural land, and achieved nitrite reduction rate of 36.08 mg NO_2^--N L^{-1} d^{-1} after 217 days. Hu et al. (2009) also successfully enriched DAMO microorganisms from mixed sediment and sludge, and achieved nitrite reduction rate of 28 mg NO_3^--N L^{-1} d^{-1} after 320 days. Vaksmaa et al. (2017) used paddy field soil as inoculum to cultivated DAMO archaea, and found that nitrate removal rate reached to 18.48 mg NO_3^--N L^{-1} d^{-1} after 730 days of enrichment. In continuous operation, since about 48% of produced biomass was washed away from the reactors, nitrogen removal rate significantly decreased from 37.8 g N m^{-3} d^{-1} to about 16.0 g N m^{-3} d^{-1} (Kampman et al., 2012). Therefore, rapid accumulation and then effective retention of enough DAMO microorganisms in bioreactor are critical for the application of DAMO processes in wastewater treatment.

Different from conventional denitrification relying on soluble organics, such as methanol (Lu et al., 2014; Torresi et al., 2017), the biomass growth and nitrogen removal of DAMO microorganisms depend on gaseous substrate—methane. Due to limited aqueous solubility (about 22 mg CH$_4$ L^{-1} at 1 atm and 20 °C) (Yamamoto et al., 1976), the low availability of methane to DAMO microorganisms could greatly limit biomass growth and nitrogen removal performance. In previous studies, membrane biofilm reactor (MBfR) has been developed to provide methane directly to biofilm containing DAMO and Anammox microorganisms that grew on the outer surface of gas-permeable hollow fiber membrane (Shi et al., 2013). Thus, high-level nitrogen removal was achieved in both sidestream and mainstream wastewater through integrating DAMO and anammox in MBfR (Xie et al., 2017; Xie et al., 2018). However, biomass retention capacity of MBfR highly depends on surface area of membrane, as the biofilm mainly grow on the outer surface of hollow fiber. To retain as many slow-growing microorganisms as possible, MBfR usually requires high specific surface area of membrane (up to 2222 m^2 m^{-3}) (Shi et al., 2013), which could significantly increase the capital cost. Besides, biofilm formation of slow-growing microorganisms is quite time consuming, resulting in about 2 years for successful startup of MBfR (Xie et al., 2017; Xie et al., 2018). Even worse, once the mature biofilm containing DAMO and Anammox microorganisms in MBfR was detached and transferred into a sequencing batch reactor, nitrogen removal performance significantly decreased from about 123 mg N L^{-1} d^{-1} to only 12 mg N L^{-1} d^{-1} (Fu et al., 2017; Fu et al., 2019). Therefore, practical application of DAMO and Anammox in wastewater treatment still face great challenges.

Therefore, the key objectives of this study are: (1) to facilitate rapid co-enrichment of DAMO and Anammox microorganisms; (2) to develop a novel Membrane Aerated Membrane Bioreactor (MAMBR), which is equipped with gas permeable membrane for efficient methane delivery and ultrafiltration membrane for complete biomass retention; (3) to investigate the feasibility of the MAMBR coupling DAMO and Anammox for nitrogen removal. To achieve the above research objectives, high concentration of mixed sludges from various environments

was fed with methane, ammonium and nitrate for fast enriching the co-cultures. The role of methane membrane aeration on activities of DAMO microorganisms was investigated through batch tests. Finally, the MAMBR was fed with synthetic wastewater containing ammonium and nitrite with the total nitrogen of 5000 mg N L^{-1} continuously. The hydraulic retention time (HRT) of MAMBR during the application phase was stepwise decreased from 8.3 to 2.0 days, and total nitrogen loading rate was increased from 600 to 2500 mg N L^{-1} d^{-1}. The performance of bioreactor was monitored by chemical analysis. The microbial community dynamics of bioreactor was investigated by 16S rRNA amplicon and fluorescence *in situ* hybridization (FISH).

2. Materials and methods

2.1. Inoculum and enrichment

The inoculum was a mixture of return activated sludges from the Wenchang wastewater treatment plant (200 mL), sediments from farmland ditch (200 mL) and anaerobic granular sludges treating paper mill wastewater (400 mL). The mixed sludges with the volatile suspended solids (VSS) of 18.70 g L^{-1} were incubated in a sequencing batch reactor (SBR) with a total volume of 2.8 L and a working volume of 2.0 L (Fig. 1a). A gas mixture (CH$_4$/CO$_2$: 95%/5%) was flushed into the reactor once a day and the pressure in the reactor headspace was kept to above 1.1 atm. The frequency of methane feeding to the reactor increased to three times a day on Day 186. The reactor was constantly mixed with magnetic mixers at 300 rpm operating at 35 ± 1.0 °C. And pH was controlled between 7.0 and 7.5 with 1 M HCl solution. Every month the reactor was settled for 1.0 h, and then 500 mL supernatant was replaced with fresh medium. The compositions of fresh medium were showed in Supplementary Information (SI). In addition, ammonium and nitrate were added by injection of concentrated anaerobic stock solutions, which made the enrichment reactor contain the nitrogen concentrations of 50–200 mg NH$_4^+$-N L^{-1} and 50–200 mg NO$_3^-$-N L^{-1}.

2.2. MAMBR setup

The MAMBR with 2.5 L of total volume and 2.0 L of working volume was equipped with pressurized gas-permeable membrane module for efficient methane delivery and ultrafiltration membrane module for complete biomass retention (Fig. 2). The membrane aeration module consisted of 256 hollow-fibers (20 M 1500 A, Mitsubishi, Ltd., Japan) with a length of 18 cm and an inner/outer fiber diameter of 180/280 μm. The total surface area of hollow-fibers membrane was 0.04 m^2 and the specific surface area of MAMBR was 20 m^2 m^{-3}. The gas (CH$_4$/CO$_2$: 95%/5%) pressure in membrane lumen was manually adjusted at 100 kPa by a pneumatic pressure relief valve (GENTEC, China) giving rise to the methane flux of 0.05 mmol CH$_4$ m^{-2} s^{-1}. The ultrafiltration membrane module with a total surface of 0.2 m^2 and a pore size of 0.05 μm (PVDF, Tianjin Motimo Membrane Technology Co., Ltd., China) was operated in cycles, with 1 min of membrane relaxation and 9 min of permeation for biomass separation. A pressure sensor (YY-YL100, China) provided the relaxation and the permeation transmembrane pressures transmitting the data to the computer system (Fig. 2).

2.3. Batch tests

The enrichment sludge was completely mixed and divided equally into two MAMBRs described in Section 2.2 (MAMBR-I and MABR-II) (Fig. 1b and c). Both reactors were operated under a fed-batch mode to investigate the effect of membrane aeration on DAMO microorganisms. Methane (CH$_4$/CO$_2$: 95%/5%) were supplied to the reactor through gas permeable membrane. Ammonium and nitrate were supplied by injection of concentrated anaerobic stock solutions. Initially, MABR-I was flushed methane three times a day. And then the membrane aeration to
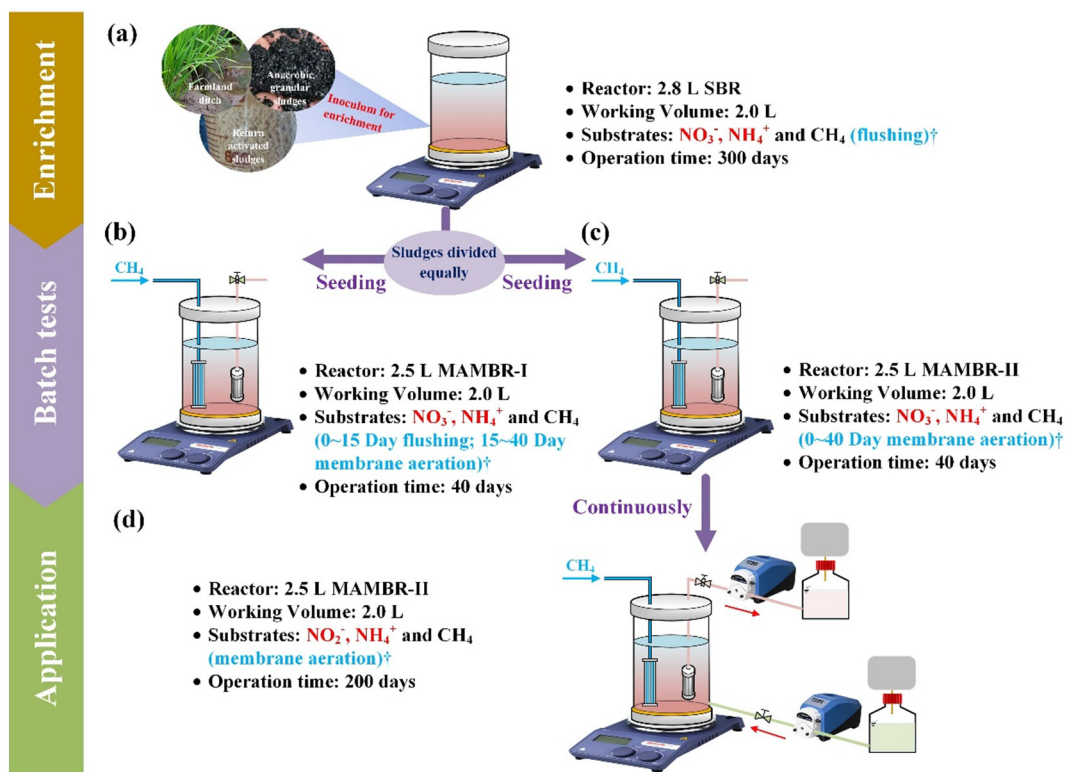
Fig. 1. Experiment procedures from enrichment to application of DAMO and Anammox († the method of methane supply to reactor).

feed methane started from Day 15 in MAMBR-I, while methane membrane aeration was carried out in MAMBR-II during whole test (Fig. 1b and c).

2.4. Continuous operation of MAMBR

After the batch tests, the MAMBR-II was continuously used to treat the synthetic wastewater containing ammonium and nitrite which mimicked the effluent from partial nitration process (Fig. 1d). Methane was continuously supplied through gas-permeable membrane module to MAMBR with the methane flux of 0.05 mmol CH_4 m^{-2} s^{-1} (Fig. 2). The initial nitrogen loading rate was 600 mg N L^{-1} d^{-1} and nitrite to ammonium ratio of 1.06 was based on the stoichiometry of DAMO and Anammox equations with the assumption that all the nitrite would be removal by the Anammox (Xie et al., 2017). The nitrogen loading rate increased to 1030 mg N L^{-1} d^{-1} on Day 9 of application, then the nitrite to ammonium in influent ratio increased to 1.12 on Day 15 and further to 1.19 on Day 22 of application to avoid the accumulation of ammonium in effluent. And then the nitrogen loading rate was stepwise increased to 1442 mg N L^{-1} d^{-1}, 2000 mg N L^{-1} d^{-1} and 2500 mg N L^{-1} d^{-1} to investigate the nitrogen removal capacity of MAMBR. The influent nitrite to ammonium ratio was reduced from 1.19 to 1.17 on Day 155 of application to achieve satisfactory effluent quality with TN below 10 mg N L^{-1} (Table 1).
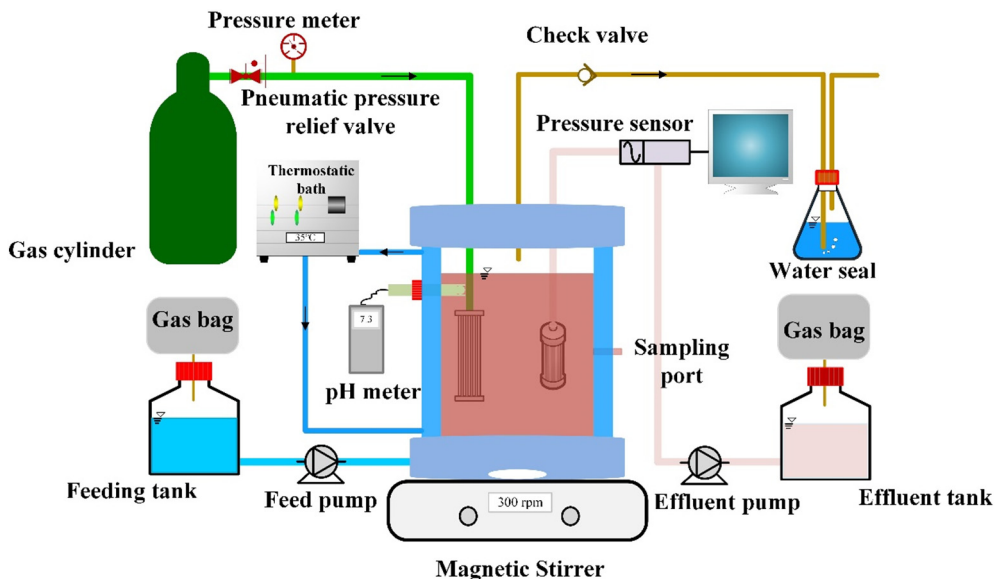

Fig. 2. Schematic diagram of MAMBR.

Table 1
Operating conditions of the MAMBR-II.

Operations	Influent (mg N L^{-1})		Total nitrogen loading rate (mg N L^{-1} d^{-1})	HRT (d)	Ratio of nitrite to ammonium in influent
	NO$_2^-$	NH$_4^+$			
Day 0–8	2572.8	2427.2	600	8.3	1.06
Day 9–14	2572.8	2427.2	1030	4.8	1.06
Day 15–21	2641.5	2358.5	1030	4.8	1.12
Day 22–56	2716.9	2283.1	1030	4.8	1.19
Day 57–78	2716.9	2283.1	1442	3.5	1.19
Day 79–125	2716.9	2283.1	2000	2.5	1.19
Day 126–154	2716.9	2283.1	2500	2.0	1.19
Day 155–200	2695.9	2304.1	2500	2.0	1.17

2.5. Analytical methods

Liquid samples from bioreactor were taken on a daily basis, and filtered with 0.22 μm sterile Millipore filter. The concentrations of NO$_3^-$-N, NO$_2^-$-N and NH$_4^+$-N in the samples were measured with Lachat QuikChem8500 Flow Injection Analyzer (Lachat Instrument, Milwaukee, WI). Based on the concentrations of NO$_3^-$-N, NO$_2^-$-N and NH$_4^+$-N, the determination of reaction rates was calculated. Every month 10 mL sludge samples were taken from the system to measure the biomass concentration (Walter, 1961). Gas samples of 1 mL were collected with gas-tight glass syringes through a rubber septum on the top of the bioreactor. Methane, carbon dioxide and dinitrogen gas were measured on a gas chromatograph (7890, Agilent, USA). The nitrogen removal rates during the enrichment and application periods were calculated according to the stoichiometries of reactions described in SI. The methane consumption and dinitrogen gas production rate were calculated on the basis of the nitrogen removal rate by key microbial functional groups. The nitrogen conversion rates by DAMO archaea, DAMO bacteria and Anammox bacteria were calculated as described in our previous study (Xie et al., 2017) (refer to SI). To further get a deeper insight into the temporal and composition changes of the microbial community during the enrichment and application, the sequences of 16S rRNA gene with excellent phylogenetic markers were taxonomically classified into some different Operational Taxonomic Units (OTUs) by > 97% similarity level. Then the relative abundance of OTUs was calculated at genus level for different periods. The DNA was extracted from the sludge sample using the Mag-Bind Soil DNA kit (OMEGA, E.Z.N.A.™) following the manufacturer's instructions. After extracting the bulk DNA, amplicon sequencing was performed with Illumina Mi-seq using primer sets 926_F and 1392_R (Klindworth et al., 2013) targeting the V6–8 region of 16S rRNA according to previous description (Engelbrektson et al., 2010) (refer to SI). The sludge samples taken from the bioreactors were fixed with 4% (v/v) paraformaldehyde and subjected to FISH analyses. The procedures of FISH and the probes used are detailed in SI.

3. Results

3.1. Simultaneous enrichment of DAMO and Anammox microorganisms

The nitrogen removal rate of the enriched sludge and biomass concentration are shown in Fig. 3a. Initially, the maximum nitrate removal rate could reach at about 90 mg NO$_3^-$-N L^{-1} d^{-1}, which could be heterotrophic denitrification supported by organics from inoculum. Then nitrate removal rate dropped shapely to 2.7 mg NO$_3^-$-N L^{-1} d^{-1} on Day 71. While the similar trend observed in biomass from the initial value of 18.71 g VSS L^{-1} to 9.12 g VSS L^{-1}, indicating that the unfavorable environment for the initial microbial community leading to function loss and cell lysis. After 145 days acclimation, gradual recovery of biomass was observed with significant increase in the
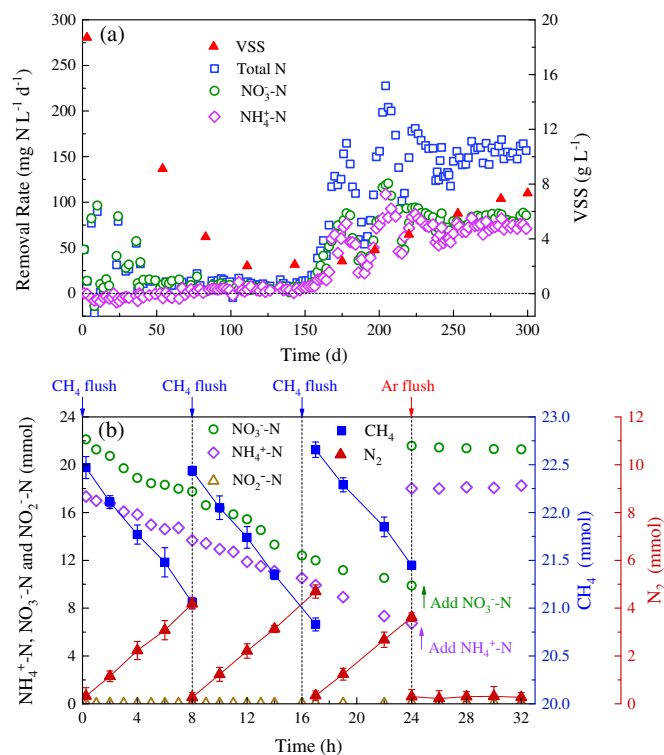

Fig. 3. The performance of the enriched sludge, (a) nitrogen removal rate and biomass concentration; (b) the profiles of the ammonium, nitrate and methane consumption and the dinitrogen gas production on Day 266 of enrichment.

ammonium and nitrate removal rate. After the maximum nitrogen removal rate of 76.68 mg NH$_4^+$-N L^{-1} d^{-1} and 87.91 mg NO$_3^-$-N L^{-1} d^{-1} was achieved on Day 178, the nitrogen removal rate sharply decreased and then to 22.8 mg NH$_4^+$-N L^{-1} d^{-1} and 36.3 mg NO$_3^-$-N L^{-1} d^{-1} on Day 186. From Day 187, the frequency of flushing methane to the reactor was increased from one to three times per day. As a result, the nitrogen removal rate quickly recovered to 65.6 mg NH$_4^+$-N L^{-1} d^{-1} and 79.3 mg NO$_3^-$-N L^{-1} d^{-1}, and then it was stable at around 75 mg NH$_4^+$-N L^{-1} d^{-1} and 85 mg NO$_3^-$-N L^{-1} d^{-1}. The performance of the enriched sludge recovered through increasing methane feeding frequency, indicating that methane availability could be the limiting factor for DAMO activity.

To elucidate the reaction mechanism and bioactivity of functional microorganisms in the enriched sludge, the profiles of the ammonium, nitrate and methane consumption and the dinitrogen gas production on Day 266 were shown (Fig. 3b) and the mass balance during 0–24 h is summarized in Table S1. During this experiment, there was no nitrite accumulation. The simultaneous consumption of ammonium, nitrate and methane suggested that both Anammox and DAMO microorganisms were present at the enriched sludge (Fig. 3b). In the case of neglecting biomass production, the nitrate removal rate by DAMO archaea of 14.99 mmol d^{-1} was calculated according to the nitrate production rate by Anammox bacteria and the measured nitrate removal rate in liquor (Table S1). The nitrite removal rate by DAMO bacteria was 0.99 mmol d^{-1} based on mass balance. The measured methane consumption rate and dinitrogen gas production rate were 4.23 mmol d^{-1} and 11.57 mmol d^{-1}, respectively, comparing very well with the respective calculated rates, with the mass balance errors of 2.63% (Table S1). After argon gas flush at 24 h, the nitrate reduction and ammonium oxidation almost completely stopped without methane supply (Fig. 3b). These results indicated that the coupling of DAMO and Anammox processes could well describe the methane and nitrogen conversions in the enriched sludge.
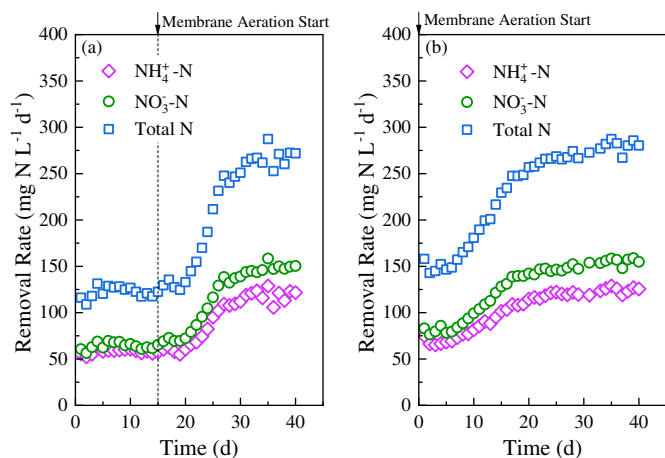
Fig. 4. Effect of membrane aeration on nitrogen removal rate. (a) Membrane aeration started from Day 15 in MAMBR-I; (b) Membrane aeration started from Day 0 in MAMBR-II.

3.2. Enhanced nitrogen removal by membrane aeration

The enriched sludges were completely mixed and divided equally into two MAMBRs (MAMBR-I and MAMBR-II), which were operated under a fed-batch mode. From Day 0 to 15, methane was supplied to MAMBR-I with traditional flush aeration. During this period the ammonium and nitrate removal rate was stable at about $55\,mg\,N\,L^{-1}\,d^{-1}$ and $65\,mg\,N\,L^{-1}\,d^{-1}$, respectively (Fig. 4a). When methane was provided through membrane aeration module for MAMBR-I on Day 15, the nitrogen removal rate significantly increased and reached the rate of $128.8\,mg\,NH_4^+-N\,L^{-1}\,d^{-1}$ and $143.5\,mg\,NO_3^--N\,L^{-1}\,d^{-1}$ on Day 35. Then the nitrogen removal rate remained at about $121\,mg\,NH_4^+-N\,L^{-1}\,d^{-1}$ and $150\,mg\,NO_3^--N\,L^{-1}\,d^{-1}$.

Unlike the MAMBR-I, methane was provided to MAMBR-II through membrane aeration during the whole test. Although the biomass concentration in MAMBR-II was only half of that in the enrichment reactor, the nitrogen removal rate achieved the similar value as enrichment reactor at $74.9\,NH_4^+-N\,L^{-1}\,d^{-1}$ and $83.1\,mg\,NO_3^--N\,L^{-1}\,d^{-1}$ in MAMBR-II on Day 1 (Fig. 4b). During Days 0–15, the ammonium and nitrate removal rate of MAMBR-II significantly increased comparing to the same period in MAMBR-I under the condition of methane membrane aeration and reached to the maximum value of $126.9\,mg\,NH_4^+-N\,L^{-1}\,d^{-1}$ and $158.8\,mg\,NO_3^--N\,L^{-1}\,d^{-1}$ on Day 39.

Since both MAMBR-I and MAMBR-II were inoculated with the equal mixed cultures from enrichment reactor, the difference of nitrogen removal during Days 0–15 suggested that membrane aeration played an important role in enhancing the activity of DAMO microorganisms. However, after methane was supplied through membrane aeration on Day 15, the nitrogen removal rate in MAMBR-I increased significantly and reached to the same level as in MAMBR-II.

3.3. Nitrogen removal performance of the MAMBR

After above tests, the MAMBR-II was changed to continuously feed with synthetic wastewater containing ammonium and nitrite to mimic the effluent from partial nitritation. The total nitrogen loading rate was $600\,mg\,N\,L^{-1}\,d^{-1}$ and nitrite to ammonium ratio in influent was kept at 1.06. Initially, both the ammonium and nitrate in effluent decreased rapidly from $76.2\,mg\,NH_4^+-N\,L^{-1}$ and $139.4\,mg\,NO_3^--N\,L^{-1}$ to $0.2\,mg$ $NH_4^+-N\,L^{-1}$ and $48.2\,mg\,NO_3^--N\,L^{-1}$, and the total nitrogen removal reached $580.5\,mg\,N\,L^{-1}\,d^{-1}$ on Day 8 (Fig. 5). Then, the total nitrogen loading rate was increased to $1030\,mg\,N\,L^{-1}\,d^{-1}$. The concentration of ammonium and nitrate in effluent increased to $105.4\,mg\,NH_4^+-N\,L^{-1}$ and $65.4\,mg\,NO_3^--N\,L^{-1}$, while nitrite in effluent was at a non-detectable level. In order to prevent ammonium further accumulating, the

ratio of nitrite to ammonium in the influent was increased to 1.12 on Day 15, and further increased to 1.19 on Day 22. As a result, the concentration of effluent ammonium significantly dropped to $0.18\,mg$ $NH_4^+-N\,L^{-1}$ on Day 30. As the experiment continued, the nitrate in effluent gradually decreased to $39.6\,mg\,NO_3^--N\,L^{-1}$, and the total nitrogen removal rate was $1022.4\,mg\,N\,L^{-1}\,d^{-1}$.

The TN loading rate increased to $1442\,mg\,N\,L^{-1}\,d^{-1}$ on Day 57. The concentration of effluent nitrite remained stable below detectable level, while the ammonium concentration in effluent increased to $41.3\,mg\,NH_4^+-N\,L^{-1}$ on Day 64, but rapidly dropped to $0.18\,mg\,NH_4^+-N\,L^{-1}$. The ammonium and nitrite removal rate were $659.8\,mg\,N\,L^{-1}\,d^{-1}$ and $785.1\,mg\,N\,L^{-1}\,d^{-1}$, respectively. The effluent nitrate increased $92.3\,mg\,NO_3^--N\,L^{-1}$ on Day 69, and then was gradually stable at about $50\,mg\,NO_3^--N\,L^{-1}$ during Day 73–78. The results indicated that the activity of DAMO archaea increased significantly. From Day 79, the HRT was decreased to 2.5 days with the TN loading rate of $2000\,mg\,N\,L^{-1}\,d^{-1}$. The ammonium and nitrate concentration in effluent fluctuated, reaching $53.5\,mg\,N\,L^{-1}$ and $166.3\,mg\,N\,L^{-1}$ on Day 103, respectively. And then the effluent ammonium and nitrate decreased to $0.7\,mg\,NH_4^+-N\,L^{-1}$ and $49.7\,mg\,NO_3^--N\,L^{-1}$, with the total nitrogen removal rate of $1979.5\,mg\,N\,L^{-1}\,d^{-1}$ on Day 125.

From Day 126, the HRT was further shortened to 2 days and the TN loading rate was $2500\,mg\,N\,L^{-1}\,d^{-1}$. The ammonium and nitrate concentration in effluent increased rapidly to $78.9\,mg\,N\,L^{-1}$ and $127.3\,mg\,N\,L^{-1}$, respectively, while nitrite was also consumed completely without accumulation. After Day 148, the ammonium and nitrate concentration in effluent were stable at about $0\,mg\,NH_4^+-N\,L^{-1}$ and $40\,mg\,NO_3^--N\,L^{-1}$. The nitrate was the main nitrogen component in the effluent. In order to further achieve complete nitrogen removal, the nitrite to ammonium ratio in influent was decreased from 1.19 to 1.17 on Day 155, while maintaining the TN loading rate of $2500\,mg\,N\,L^{-1}\,d^{-1}$. The ammonium in effluent increased to $9.0\,mg$ $NH_4^+-N\,L^{-1}$ on Day 159, but soon decreased to below $0.1\,mg\,NH_4^+-N\,L^{-1}$ on Day 165. And the effluent nitrate gradually decreased to $6.49\,mg\,NO_3^--N\,L^{-1}$ on Day 190. Nitrite concentration in the effluent remained at non-detectable level. After that, the MAMBR-II achieved a steady-state with the ammonium and nitrite removal rate of $1152\,mg$ $NH_4^+-N\,L^{-1}\,d^{-1}$ and $1347\,mg\,NO_2^--N\,L^{-1}\,d^{-1}$. The effluent only contained nitrate below $7\,mg\,NO_3^--N\,L^{-1}$, with a total nitrogen removal efficiency of $> 99.87\%$.

3.4. Microbial community dynamics in the enrichment and application

Sequence analysis and FISH were used to investigate the microbial community dynamics during the enrichment and application (Fig. 6). A large number of methanogens were detected in the inoculum, accounted for 30.58% of whole microbial community, including genus *Methanothrix*, *Methanolinea*, *Methanomassiliicoccus*, *Methanosalsum* and so on. The abundance of methanogens increased to 41.98% on Day 31, but gradually decreased to 3.61% on Day180, and finally disappeared after Day 272 of enrichment. Genus *Denitratisoma*, a typical group of denitrifying bacteria (Fahrbach et al., 2006; Ma et al., 2015), was also detected in the inoculum with abundance of 2.56%, then increased to 8.06% on Day 31, but died out rapidly due to depletion of organic carbon sources during enrichment. There were only 26 sequences of *Ca.* Methanoperedens in the inoculum (Table S2), which is DAMO archaea performing nitrate-driven anaerobic methane oxidation (Haroon et al., 2013). The relative abundance of *Ca.* Methanoperedens was over 1.10% on Day 59 and gradually increased to 3.25% on Day 92, then to 37.32% significantly on Day 180 of enrichment. There were 314 sequences in the seed sludges allocated into three genera, *Ca.* Kuenenia, *Ca.* Anammoxoglobus and *Ca.* Brocadia, which are known Anammox bacteria (Kartal et al., 2010; Kartal et al., 2011) (Table S2). The relative abundance of *Ca.* Kuenenia increased to 8.82% on Day 180 of enrichment, while *Ca.* Anammoxoglobus and *Ca.* Brocadia gradually disappeared from the enrichment reactor. There were only 8 sequences in the seed
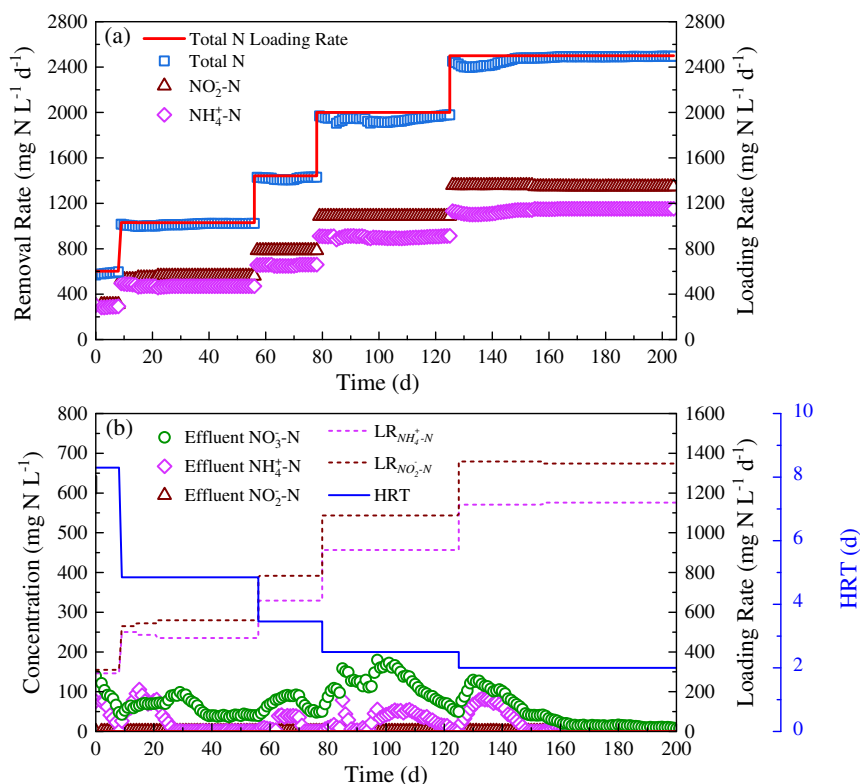
Fig. 5. Performance of the MAMBR-II during 200 days of operation. (a) Total nitrogen loading rate and removal rate, ammonium, nitrite and nitrate removal rate; (b) Ammonium and nitrite loading rate; ammonium, nitrite and nitrate concentration in effluent.

sludges grouping into the genus *Ca.* Methylomirabilis (Table S2), which vests in phylum *NC10* performing nitrite-driven anaerobic methane oxidation (Ettwig et al., 2010). From Day 180, the relative abundance of genus *Ca.* Methylomirabilis slightly increased from 0.98% to 1.78% on Day 242. At the end of enrichment, microbial community of co-cultures composed of 56.51% *Ca.* Methanoperedens followed by 9.69% *Ca.* Kuenenia and 2.28% *Ca.* Methylomirabilis on Day 300 of enrichment.

During application stage, MAMBR-II was continuously fed with synthetic wastewater containing ammonium and nitrite. The increasing loading rate of ammonium and nitrite stimulated the growth of Anammox bacteria, so the relative abundance of *Ca.* Kuenenia significantly increased to 44.99% on Day 182. In contrast, the relative abundance of *Ca.* Methanoperedens gradually decreased to 36.44% on Day 182. After that, the relative abundance of DAMO archaea and Anammox bacteria also maintained stable at about 37% and 45%, respectively (Fig. 6a). As a result, the sludge flocs gradually turned red with the increase abundance of Anammox bacteria (Fig. 6b and c). The sequence analysis results are broadly supported by FISH images acquired (Fig. 6d and e). DAMO archaea were the dominant microorganisms in the enrichment process fed with nitrate and ammonium, following by the Anammox bacteria, while the DAMO bacteria could hardly be detected in the enrichment (Fig. 6d). In contrast, MAMBR-II was continuously fed with nitrite and ammonium, which stimulated the growth of Anammox bacteria. Thus, abundance of Anammox significantly increased (Fig. 6e). In addition, the co-occurrence of methane and nitrite in MAMBR-II provided an environment suitable for the development of DAMO bacteria. As a result, DAMO bacteria were also detected with relative low abundance (Fig. 6e).

4. Discussion

The difficulty for DAMO research and application is the long enrichment period in the range of 217 to 600 day (Table S3). In this study,

the co-cultures of DAMO archaea, DAMO bacteria and Anammox bacteria were fast enriched using high concentration of mixed sludges from various environments. The relative abundance of DAMO archaea, DAMO bacteria and Anammox bacteria was 40.87%, 1.31% and 9.94% on Day 214 of enrichment. The higher nitrogen removal rate of 76.7 mg NH_4^+-N L^{-1} d^{-1} and 87.9 mg NO_3^--N L^{-1} d^{-1} was achieved after 178 days enrichment. To our best knowledge, this is the first time that the DAMO archaea with such high and stable performance were enriched in such shorter period (Table S3). Inoculum resources are an important factor for successful enrichment of DAMO microorganisms (He et al., 2015). Besides Anammox granules (Zhu et al., 2011), freshwater sediments (Bhattacharjee et al., 2016), paddy soil (Zhu et al., 2011) and peatland (Zhu et al., 2012) were also reported as inoculum for DAMO enrichment. In this study, the activated sludge, sediments from farmland ditch and anaerobic granular sludge treating paper mill wastewater were collected and mixed as the inoculum. Collecting inoculum from various environment ensures the broad microbial community and high biomass concentration of inoculum (18.70 g VSS L^{-1}) provides more target microbes for enrichment, which offer the opportunity for accelerated whole process. As shown in Table S2, the sequences numbers of DAMO archaea, DAMO bacteria and Anammox bacteria were 26, 8 and 314 in the inoculum sludges, respectively. The large number of non-target microbes were selectively died out due to specific culture conditions for DAMO and Anammox microorganisms (Fig. 6a), and large deletion in biomass was observed from 18.7 to 2.02 g VSS L^{-1} in the first 111 days (Fig. 3b). After that, biomass concentration gradually increased with the increase of nitrogen removal rate, and achieved 7.34 g VSS L^{-1} at the end of enrichment (300 days).

During the enrichment, nitrate removal rate by DAMO archaea significantly dropped to 37.6 mg NO_3^--N L^{-1} d^{-1} after reaching the maximum value of 106.8 mg NO_3^--N L^{-1} d^{-1}. Activity of DAMO archaea quickly returned to 108.5 mg NO_3^--N L^{-1} d^{-1} through increasing feed of methane. Unexpected decline of DAMO activities
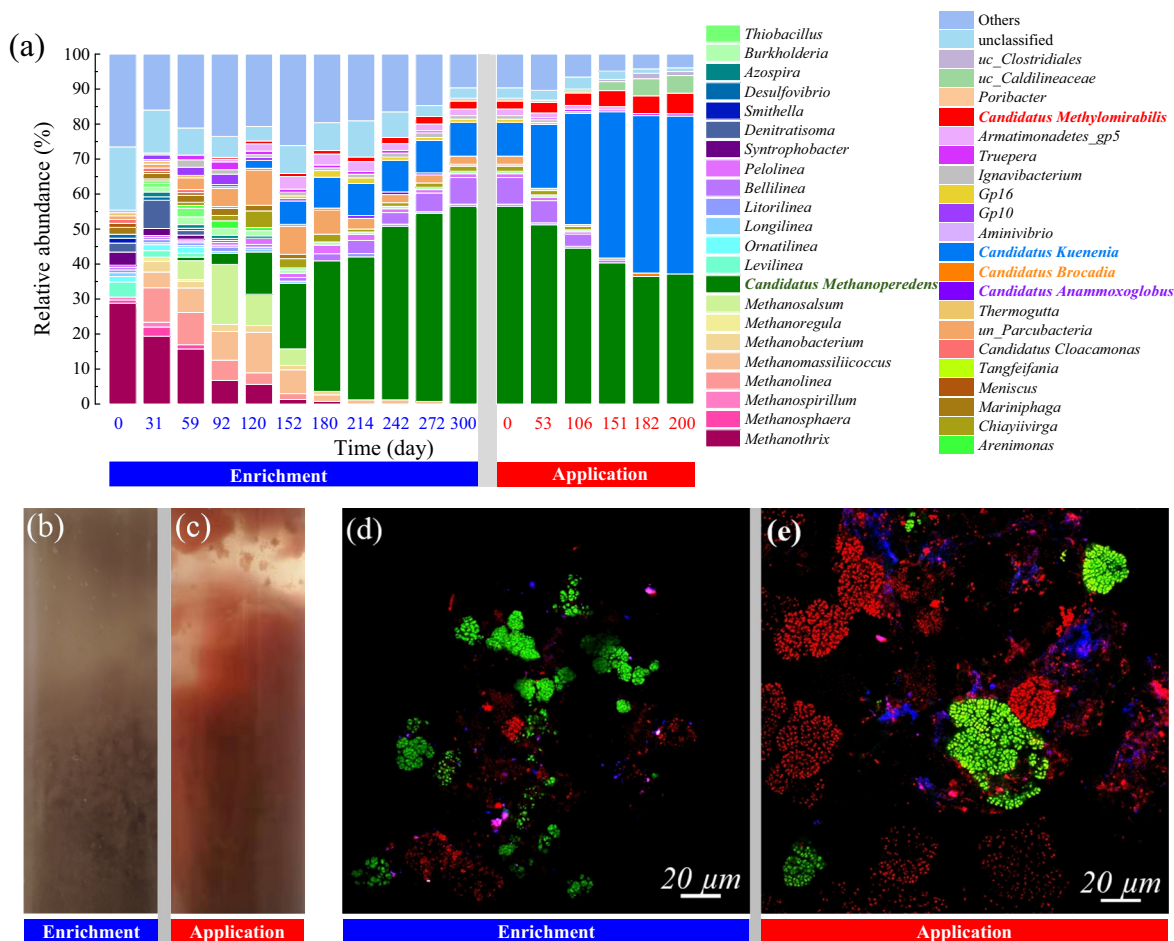
Fig. 6. Microbial community dynamics during enrichment and application. Genus level distributions of microbial population (a), Taxa with relative abundance of > 0.5% in the culture were shown, while the remaining taxa were grouped into 'Others'. The prefix *uc_* indicates unclassified taxa. The morphology of the sludge flocs photographed on Day 300 day of enrichment (b) and on Day 200 of application (c); FISH micrographs of the sample collected on Day 300 of enrichment (d) and on Day 200 of application (e), DAMO archaea (green), DAMO bacteria (blue) and anammox bacteria (red). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

during the enrichment has been reported in previous studies (Ding et al., 2014; Hu et al., 2011; Kampman et al., 2012). The recovery of DAMO activities was also achieved by external circulation which increased the methane supply through enhancing the gas-liquid contact (Ding et al., 2014), but the reactor activity decreased to a low level again. With the increase of DAMO activity, the methane consumption rate of DAMO microorganisms will go up to a critical level, which could lead to insufficient methane mass transfer into the sludge flocs, especially with high concentration of biomass. Thus, methane availability could be the limiting factor for further improvement of DAMO activities. In this work, gas-permeable membrane was used to supply gaseous methane continuously to suspended sludge containing DAMO archaea and DAMO bacteria. Methane is delivered by diffusion through the wall of gas-permeable membrane without bubble formation, and capacity of methane supply is controlled through adjustment of methane pressure inside the membrane (Hwang et al., 2009). Consequently, the nitrate removal rate by DAMO archaea increased to $181.5 \, mg \, N \, L^{-1} \, d^{-1}$ after membrane aeration applied.

As DAMO and Anammox microorganisms are well-known slow-growing with doubling time over 10 days, the retention of enough DAMO and Anammox biomass in bioreactor is critical for the application of DAMO processes in wastewater treatment. Washout of the DAMO bacteria from reactor caused the significant decrease of the nitrogen removal rate from $37.8 \, mg \, NO_2^- \text{-} N \, L^{-1} \, d^{-1}$ to about $16.0 \, mg$ $NO_2^- \text{-} N \, L^{-1} \, d^{-1}$ (Kampman et al., 2012). In this work, complete

biomass retention was achieved using ultrafiltration membrane. The biomass was completely separated with effluent, gradually accumulated in the reactor and reached $8.67 \, g \, VSS \, L^{-1}$ at end of operation (Fig. S1). As a result, apparent specific activity of $288 \, g \, N \, kg^{-1} \, VSS \, d^{-1}$ was obtained in coupled DAMO and Anammox process, which is comparable with $210–350 \, g \, N \, kg^{-1} \, VSS \, d^{-1}$ in Anammox processes (Allegue et al., 2018; Tang et al., 2011). Therefore, another significant advantage offered by MAMBR is complete biomass retention, which enables DAMO and Anammox to handle large volumetric flows and loading capacities.

In our previous study, membrane biofilm reactors (MBfRs) were developed, which used gas-permeable hollow fiber membranes to provide methane and to offer surface supporting biofilm attachment for the growth of DAMO and anammox organisms (Xie et al., 2017; Xie et al., 2018). Through integrating DAMO and anammox in MBfR, high-level nitrogen removal was obtained in both sidestream ($1.03 \, kg \, N \, m^{-3} \, d^{-1}$) and mainstream ($0.2 \, kg \, N \, m^{-3} \, d^{-1}$) wastewater. However, successful startup of MBfR experienced about 2 years (Table 2), as biofilm formation of slow-growing microorganisms is quite time consuming. Even worse, once the mature biofilm cultivated in MBfR was detached and transferred into a sequencing batch reactor, nitrogen removal performance significantly decreased from about $123 \, mg \, N \, L^{-1} \, d^{-1}$ to only $12 \, mg \, N \, L^{-1} \, d^{-1}$ (Fu et al., 2017; Fu et al., 2019). Consequently, such a long start-up period would hinder the practical application of DAMO and Anammox. In this work, MAMBR is developed to combine i)

Table 2
The performance of present processes coupling DAMO and Anammox processes for nitrogen removal.

Reactor	Biomass	A/V (m² m⁻³)[c]	Influent (mg N L⁻¹)			Removal rate (mg N L⁻¹ d⁻¹)			Volumetric nitrogen removal rate (g N L⁻¹ d⁻¹)	Superficial nitrogen removal rate (g N m⁻² d⁻¹)[e]	Methane consumption rate (mg L⁻¹ d⁻¹)[f]	Operation time (d)	Reference
			NH₄⁺-N	NO₃⁻-N	NO₂⁻-N	NH₄⁺-N	NO₃⁻-N	NO₂⁻-N					
MBfR[a]	biofilm	2222	300	600	0	60	190	NA	0.25	0.11	112.96	720	(Shi et al., 2013)
MBfR	biofilm	2222	400	0	NA[d]	268	614	NA	0.88	0.40	336.73	453	(Cai et al. 2015)
MBfR	biofilm	491	470	0	560	470	NA	560	1.03	2.10	61.39	627	(Xie et al., 2017)
MBfR	biofilm	491	22	0	29.5	128	NA	NA	0.28	0.57	24.22	730	(Xie et al., 2018)
MAMBR[b]	floc	20	2304	0	2696	1152	NA	1347	2.50	124.83	139.53	200	This study

[a] Membrane biofilm reactor.
[b] Membrane aerated membrane bioreactor.
[c] Aeration membrane specific surface area.
[d] Not available.
[e] Superficial nitrogen removal rate was calculated by dividing the volumetric nitrogen removal rate by the surface area of aeration membranes.
[f] Methane consumption rate based on the nitrogen removal rate and reaction stoichiometry.

membrane aeration for efficient methane supply and ii) membrane filtration for complete separating the slow-growing microorganisms from the mixed liquor. After inoculated enriched sludges from parent reactor, the MAMBR achieved a steady-state with a total nitrogen removal rate of $2.50 \, \text{kg N m}^{-3} \, \text{d}^{-1}$ within just 200 days. To our knowledge, such high nitrogen removal performance through integrating DAMO and Anammox process has never been reported before, especially in such a short period. Therefore, bypassing time-consuming biofilm cultivation, MAMBR completely retained biomass of DAMO and Anammox in reactor using ultrafiltration membrane, which substantially reduced the startup time of DAMO and Anammox process.

In addition, biomass retention capacity of MBfR highly depends on surface area of membrane, as the biofilm mainly grow on the outer surface of hollow fiber. As a result, MBfR usually require high specific surface area of membrane from 491 to $2222 \, \text{m}^2 \, \text{m}^{-3}$ (Table 2) to retain as many slow-growing microorganisms as possible. Consequently, volumetric nitrogen removal rate of MBfR reached $0.25–1.03 \, \text{g N L}^{-1} \, \text{d}^{-1}$ at the cost of gas-permeable membrane. However, the large amount of hollow fiber membranes installed in MBfR not only significantly increased operation and maintenance costs, but also resulted in superficial nitrogen removal rate at low level from 0.11 to $2.10 \, \text{g N m}^{-2} \, \text{d}^{-1}$. In this work, gas-permeable membrane was used for methane delivery to bulk liquid rather than biofilm cultivation, so specific surface area $(20 \, \text{m}^2 \, \text{m}^{-3})$ of membranes in MAMBR is only 0.9%~4.1% of that in MBfR, but the superficial nitrogen removal rate of MAMBR is highest at $124.83 \, \text{g N m}^{-2} \, \text{d}^{-1}$ (Table 2). Thus, MAMBR is not limited by the surface area of hollow fiber membranes as the biomass is suspended floc rather than biofilm. What's more, the suspended biomass cultivated in the MAMBR can be easily collected and transferred into other reactors as seed inoculum, which can significantly accelerate the further startup process. Therefore, MAMBR will be convenient for scale-up and shed new light on the practical applications of coupled DAMO and Anammox process in wastewater treatment.

Currently, membrane technology has been widely applied for seawater desalination and water recycle, while membrane fouling remains as a major challenge (Meng et al., 2018). During the long-term operation of MAMBR, membrane fouling will occur on both gas-permeable membrane and ultrafiltration membrane, resulting in decrease of methane flux and reduction of permeate flux. Thus, fouling behaviors and mechanisms of DAMO and Anammox microorganisms on gas-permeable membrane and ultrafiltration membrane will be focused in our studies to develop strategies for fouling controls.

5. Conclusions

In this study, the co-cultures of DAMO and Anammox microorganisms were fast enriched and then were applicated for nitrogen removal from synthetic wastewater in a novel lab-scale MAMBR. The major outcomes and conclusions can be summarized as follows:

(1) With high biomass concentration of inoculum ($18.70 \, \text{g VSS L}^{-1}$) from various environment, DAMO and Anammox microorganisms were fast enriched and reached nitrogen removal rate of 76.7 mg NH_4^+-N $\text{L}^{-1} \, \text{d}^{-1}$ and 87.9 mg NO_3^--N $\text{L}^{-1} \, \text{d}^{-1}$ on 178 days enrichment.
(2) Nitrogen removal rates of DAMO and Anammox microorganisms were further enhanced to about 125 mg NH_4^+-N $\text{L}^{-1} \, \text{d}^{-1}$ and 158 mg NO_3^--N $\text{L}^{-1} \, \text{d}^{-1}$ within 25 days through efficient methane delivery using gas permeable membrane.
(3) The high performance of nitrogen removal from synthetic wastewater mimicking partial nitritation effluent ($2.5 \, \text{kg N m}^{-3} \, \text{d}^{-1}$) were obtained in MAMBR along with satisfactory effluent quality ($< 7 \, \text{mg TN L}^{-1}$).
(4) The membrane aerated membrane bioreactor is a practical technology for application of anammox and DAMO processes through combining membrane aeration for efficient methane supply and

membrane filtration for complete separating the slow-growing microorganisms from the mixed liquor.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, or the review of, the manuscript entitled.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2019.105107.

References

Allegue, T., Arias, A., Fernandez-Gonzalez, N., Omil, F., Garrido, J.M., 2018. Enrichment of nitrite-dependent anaerobic methane oxidizing bacteria in a membrane bioreactor. Chem. Eng. J. 347, 721–730.

Andert, J., Borjesson, G., Hallin, S., 2012. Temporal changes in methane oxidizing and denitrifying communities and their activities in a drained peat soil. Wetlands 32, 1047–1055.

Bhattacharjee, A.S., Motlagh, A.M., Jetten, M.S.M., Goel, R., 2016. Methane dependent denitrification- from ecosystem to laboratory-scale enrichment for engineering applications. Water Res. 99, 244–252.

Cai, C., Hu, S., Guo, J., Shi, Y., Xie, G.-J., Yuan, Z., 2015. Nitrate reduction by denitrifying anaerobic methane oxidizing microorganisms can reach a practically useful rate. Water Res 87, 211–217.

Chen, X., Guo, J., Shi, Y., Hu, S., Yuan, Z., Ni, B.-J., 2014. Modeling of simultaneous anaerobic methane and ammonium oxidation in a membrane biofilm reactor. Environ. Sci. Technol. 48, 9540–9547.

Chen, J., Zhou, Z., Gu, J.-D., 2015. Complex community of nitrite-dependent anaerobic methane oxidation bacteria in coastal sediments of the Mai Po wetland by PCR amplification of both 16S rRNA and pmoA genes. Appl. Microbiol. Biotechnol. 99, 1463–1473.

Czepiel, P.M., Crill, P.M., Harriss, R.C., 1993. Methane emissions from municipal wastewater treatment processes. Environ. Sci. Technol. 27, 2472–2477.

Ding, Z.-W., Ding, J., Fu, L., Zhang, F., Zeng, R.J., 2014. Simultaneous enrichment of denitrifying methanotrophs and anammox bacteria. Appl. Microbiol. Biotechnol. 98, 10211–10221.

Ding, J., Fu, L., Ding, Z.-W., Lu, Y.-Z., Cheng, S.H., Zeng, R.J., 2016. Environmental evaluation of coexistence of denitrifying anaerobic methane-oxidizing archaea and bacteria in a paddy field. Appl. Microbiol. Biotechnol. 100, 439–446.

El-Fadel, M., Massoud, M., 2001. Methane emissions from wastewater management. Environ. Pollut. 114, 177–185.

Engelbrektson, A., Kunin, V., Wrighton, K.C., Zvenigorodsky, N., Chen, F., Ochman, H., Hugenholtz, P., 2010. Experimental factors affecting PCR-based estimates of microbial species richness and evenness. ISME J. 4, 642–647.

Ettwig, K.F., Shima, S., van de Pas-Schoonen, K.T., Kahnt, J., Medema, M.H., op den Camp, H.J.M., Jetten, M.S.M., Strous, M., 2008. Denitrifying bacteria anaerobically oxidize methane in the absence of archaea. Environ. Microbiol. 10, 3164–3173.

Ettwig, K.F., van Alen, T., van de Pas-Schoonen, K.T., Jetten, M.S.M., Strous, M., 2009. Enrichment and molecular detection of denitrifying methanotrophic bacteria of the NC10 phylum. Appl. Environ. Microbiol. 75, 3656–3662.

Ettwig, K.F.; Butler, M.K.; Le Paslier, D.; Pelletier, E.; Mangenot, S.; Kuypers, M.M.M.; Schreiber, F.; Dutilh, B.E.; Zedelius, J.; de Beer, D.; Gloerich, J.; Wessels, H.J.C.T.; van Alen, T.; Luesken, F.; Wu, M.L.; van de Pas-Schoonen, K.T.; den Camp, H.J.M.O.; Janssen-Megens, E.M.; Francoijs, K.-J.; Stunnenberg, H.; Weissenbach, J.; Jetten, M.S.M.; Strous, M. Nitrite-driven anaerobic methane oxidation by oxygenic bacteria. Nature 2010;464:(543-+).

Fahrbach, M., Kuever, J., Meinke, R., Kämpfer, P., Hollender, J., 2006. Denitratisoma oestradiolicum gen. nov., sp. nov., a 17β-oestradiol-degrading, denitrifying

betaproteobacterium. Int. J. Syst. Evol. Microbiol. 56, 1547–1552.

Fu, L., Ding, J., Lu, Y.-Z., Ding, Z.-W., Bai, Y.-N., Zeng, R.J., 2017. Hollow fiber membrane bioreactor affects microbial community and morphology of the DAMO and Anammox co-culture system. Bioresour. Technol. 232, 247–253.

Fu, L., Zhang, F., Bai, Y.-N., Lu, Y.-Z., Ding, J., Zhou, D., Liu, Y., Zeng, R.J., 2019. Mass transfer affects reactor performance, microbial morphology, and community succession in the methane-dependent denitrification and anaerobic ammonium oxidation co-culture. Sci. Total Environ. 651, 291–297.

Guo, Y., Zhao, Y., Zhu, T., Li, J., Feng, Y., Zhao, H., Liu, S., 2018. A metabolomic view of how low nitrogen strength favors anammox biomass yield and nitrogen removal capability. Water Res. 143, 387–398.

Haroon, M.F., Hu, S., Shi, Y., Imelfort, M., Keller, J., Hugenholtz, P., Yuan, Z., Tyson, G.W., 2013. Anaerobic oxidation of methane coupled to nitrate reduction in a novel archaeal lineage. Nature 500, 567.

He, Z., Cai, C., Geng, S., Lou, L., Xu, X., Zheng, P., Hu, B., 2013. Mdodeling a nitrite-dependent anaerobic methane oxidation process: parameters identification and model evaluation. Bioresour. Technol. 147, 315–320.

He, Z., Cai, C., Shen, L., Lou, L., Zheng, P., Xu, X., Hu, B., 2015. Effect of inoculum sources on the enrichment of nitrite-dependent anaerobic methane-oxidizing bacteria. Appl. Microbiol. Biotechnol. 99, 939–946.

Hu, S., Zeng, R.J., Burow, L.C., Lant, P., Keller, J., Yuan, Z., 2009. Enrichment of denitrifying anaerobic methane oxidizing microorganisms. Environ. Microbiol. Rep. 1, 377–384.

Hu, S., Zeng, R.J., Keller, J., Lant, P.A., Yuan, Z., 2011. Effect of nitrate and nitrite on the selection of microorganisms in the denitrifying anaerobic methane oxidation process. Environ. Microbiol. Rep. 3, 315–319.

Hwang, J.H., Cicek, N., Oleszkiewicz, J.A., 2009. Membrane biofilm reactors for nitrogen removal: state-of-the-art and research needs. Water Sci. Technol. 60, 2739–2747.

Kampman, C., Hendrickx, T.L.G., Luesken, F.A., van Alen, T.A., Op den Camp, H.J.M., Jetten, M.S.M., Zeeman, G., Buisman, C.J.N., Temmink, H., 2012. Enrichment of denitrifying methanotrophic bacteria for application after direct low-temperature anaerobic sewage treatment. J. Hazard. Mater. 227, 164–171.

Kartal, B., Kuenen, J.G., van Loosdrecht, M.C.M., 2010. Sewage treatment with Anammox. Science 328, 702–703.

Kartal, B., Maalcke, W.J., de Almeida, N.M., Cirpus, I., Gloerich, J., Geerts, W., den Camp, H.J.M.O., Harhangi, H.R., Janssen-Megens, E.M., Francoijs, K.-J., Stunnenberg, H.G., Keltjens, J.T., Jetten, M.S.M., Strous, M., 2011. Molecular mechanism of anaerobic ammonium oxidation. Nature 479, 127–U159.

van Kessel, M.A.H.J., Stultiens, K., Slegers, M.F.W., Cruz, S.G., Jetten, M.S.M., Kartal, B., Op den Camp, H.J.M., 2018. Current perspectives on the application of N-damo and anammox in wastewater treatment. Curr. Opin. Biotechnol. 50, 222–227.

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., Gloeckner, F.O., 2013. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Res. 41.

Lu, H., Chandran, K., Stensel, D., 2014. Microbial ecology of denitrification in biological wastewater treatment. Water Res. 64, 237–254.

Ma, J., Wang, Z., He, D., Li, Y., Wu, Z., 2015. Long-term investigation of a novel electrochemical membrane bioreactor for low-strength municipal wastewater treatment. Water Res. 78, 98–110.

Martinez-Cruz, K., Sepulveda-Jauregui, A., Casper, P., Anthony, K.W., Smemo, K.A., Thalasso, F., 2018. Ubiquitous and significant anaerobic oxidation of methane in freshwater lake sediments. Water Res 144, 332–340.

Meng, S., Fan, W., Li, X., Liu, Y., Liang, D., Liu, X., 2018. Intermolecular interactions of polysaccharides in membrane fouling during microfiltration. Water Res. 143, 38–46.

Raghoebarsing, A.A., Pol, A., van de Pas-Schoonen, K.T., Smolders, A.J.P., Ettwig, K.F., Rijpstra, W.I.C., Schouten, S., Damste, J.S.S., Op den Camp, H.J.M., Jetten, M.S.M., Strous, M., 2006. A microbial consortium couples anaerobic methane oxidation to denitrification. Nature 440, 918–921.

Regmi, P., Holgate, B., Miller, M.W., Park, H., Chandran, K., Wett, B., Murthy, S., Bott, C.B., 2016. Nitrogen polishing in a fully anoxic anammox MBBR treating mainstream nitritation–denitritation effluent. Biotechnol. Bioeng. 113, 635–642.

Shen, L.-d., Liu, S., Zhu, Q., Li, X.-y., Cai, C., Cheng, D.-q., Lou, L.-p., Xu, X.-y., Zheng, P., Hu, B.-l., 2014. Distribution and diversity of nitrite-dependent anaerobic methane-oxidising bacteria in the sediments of the Qiantang River. Microb. Ecol. 67, 341–349.

Shi, Y., Hu, S.H., Lou, J.Q., Lu, P.L., Keller, J., Yuan, Z.G., 2013. Nitrogen removal from wastewater by coupling Anammox and methane-dependent denitrification in a membrane biofilm reactor. Environ. Sci. Technol. 47, 11577–11583.

Tang, C.-J., Zheng, P., Wang, C.-H., Mahmood, Q., Zhang, J.-Q., Chen, X.-G., Zhang, L., Chen, J.-W., 2011. Performance of high-loaded ANAMMOX UASB reactors containing granular sludge. Water Res. 45, 135–144.

Torresi, E., Casas, M.E., Polesel, F., Plosz, B.G., Christensson, M., Bester, K., 2017. Impact of external carbon dose on the removal of micropollutants using methanol and ethanol in post-denitrifying moving bed biofilm reactors. Water Res. 108, 95–105.

Vaksmaa, A., Guerrero-Cruz, S., van Alen, T.A., Cremers, G., Ettwig, K.F., Luke, C., Jetten, M.S.M., 2017. Enrichment of anaerobic nitrate-dependent methanotrophic 'Candidatus Methanoperedens nitroreducens' archaea from an Italian paddy field soil. Appl. Microbiol. Biotechnol. 101, 7075–7084.

Walter, W.G., 1961. Standard methods for the examination of water and wastewater (11th ed.). American Journal of Public Health and the Nations Health 51, 940.

Wang, Y., Zhu, G., Harhangi, H.R., Zhu, B., Jetten, M.S.M., Yin, C., Op den Camp, H.J.M., 2012. Co-occurrence and distribution of nitrite-dependent anaerobic ammonium and methane-oxidizing bacteria in a paddy soil. FEMS Microbiol. Lett. 336, 79–88.

Xie, G.-J., Cai, C., Hu, S., Yuan, Z., 2017. Complete nitrogen removal from synthetic anaerobic sludge digestion liquor through integrating Anammox and denitrifying anaerobic methane oxidation in a membrane biofilm reactor. Environ. Sci. Technol. 51, 819–827.

Xie, G.-J., Liu, T., Cai, C., Hu, S., Yuan, Z., 2018. Achieving high-level nitrogen removal in mainstream by coupling anammox with denitrifying anaerobic methane oxidation in a membrane biofilm reactor. Water Res. 131, 196–204.

Xu, Y.N., Tong, T.L., Chen, J.F., Li, B.X., Xie, S.G., 2018. Nitrite-dependent methane-oxidizing bacteria seasonally and spatially shift in a constructed wetland used for treating river water. Ecol. Eng. 110, 48–53.

Yamamoto, S., Alcauskas, J.B., Crozier, T.E., 1976. Solubility of methane in distilled water and seawater. J. Chem. Eng. Data 21, 78–80.

Yang, M., Guo, Q., Tong, T., Li, N., Xie, S., Long, Y., 2017. Vegetation type and layer depth influence nitrite-dependent methane-oxidizing bacteria in constructed wetland. Arch. Microbiol. 199, 505–511.

Zhou, L., Wang, Y., Long, X.-E., Guo, J., Zhu, G., 2014. High abundance and diversity of nitrite-dependent anaerobic methane-oxidizing bacteria in a paddy field profile. FEMS Microbiol. Lett. 360, 33–41.

Zhu, B., Sanchez, J., van Alen, T.A., Sanabria, J., Jetten, M.S.M., Ettwig, K.F., Kartal, B., 2011. Combined anaerobic ammonium and methane oxidation for nitrogen and methane removal. Biochem. Soc. Trans. 39, 1822–1825.

Zhu, B., van Dijk, G., Fritz, C., Smolders, A.J.P., Pol, A., Jetten, M.S.M., Ettwig, K.F., 2012. Anaerobic oxidization of methane in a minerotrophic peatland: enrichment of nitrite-dependent methane-oxidizing bacteria. Appl. Environ. Microbiol. 78, 8657–8665.