



# Gas-delivery membrane as an alternative aeration method to remove dissolved methane from anaerobically treated wastewater

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## ARTICLE INFO

### Keywords:

Dissolved methane  
Aerobic methane oxidation  
Nitrite/nitrate-dependent anaerobic methane oxidation (n-DAMO)  
Anammox  
Membrane aerated biofilm reactor (MABR)

## ABSTRACT

Dissolved methane is a hurdle for anaerobic wastewater treatment, which would be stripped into the atmosphere by conventional bubble aeration and increase the release of greenhouse gases into the environment. The high oxygen transfer efficiency and less turbulence in membrane aerated biofilm reactor (MABR) could prevent the stripping of dissolved methane. In this study, an MABR was established to remove dissolved methane aerobically in parallel to the nitrogen removal driven by the anammox process. The long-term results demonstrated that aerobic methane oxidation has a short start-up period, in which a high level (>90 %) of dissolved methane removal was achieved in 20 days. Meanwhile, the anammox-based nitrogen removal process reached a total nitrogen removal rate of ~150 mg N/L/d (0.27 g N/m<sup>2</sup>/d). In situ batch tests confirmed the active bioreactions of ammonia-oxidizing bacteria, nitrite-oxidizing bacteria, anammox bacteria and aerobic methanotrophs, while 16S rRNA gene amplicon sequencing further validated their existence. Moreover, nitrite/nitrate-dependent anaerobic methane oxidation (n-DAMO) bacteria were enriched to a relative abundance of 2.5 % on Day 372, suggesting their potential role in removing nitrogen and dissolved methane in the MABR. This study provides an alternative technology for removing dissolved methane and nitrogen in parallel from anaerobically treated wastewater.

## 1. Introduction

Wastewater treatment is undergoing a significant paradigm shift from pollutant removal to resource recovery. One of the most promising configurations for bioenergy recovery from wastewater is the conversion of organic carbons to biogas using anaerobic treatment, followed by downstream autotrophic nitrogen removal (e.g., partial nitrification and anammox, PN/A) (Liu et al., 2024; McCarty, 2018). However, up to 50 % of the produced methane is dissolved in the anaerobic effluent. The dissolved methane is easily stripped into the atmosphere, due to the turbulence caused by the downstream bubbling aeration. Methane is estimated to have 25 times higher warming effects than carbon dioxide on a 100-year horizon (Liu et al., 2014). Therefore, the stripping of dissolved methane would cause a sharp increase in the carbon footprint of wastewater treatment (Daelman et al., 2014).

Numerous technologies have been developed to solve the issue of dissolved methane in anaerobic effluent, including conventional gas stripping (Glória et al., 2016), membrane contactors (Cookney et al., 2016), and biological methane oxidation (Hatamoto et al., 2010; Liu et al., 2023b, 2020). Gas stripping and membrane contactors can be easily implemented, both of which have been demonstrated to remove dissolved methane from anaerobic effluents efficiently (Cookney et al., 2016; Sanchis-Perucho et al., 2020). However, these two approaches suffer from similar limitations. First, the energy consumption substantially increases as the dissolved methane concentration decreases. Thus, the gas stripping and membrane contactors are generally energy-intensive when complete dissolved methane removal is targeted. Moreover, the recovered gas contains highly diluted methane, which is not qualified for flaring or for further gas upgrading (Glória et al., 2016). In contrast, biological treatment of dissolved methane is more

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<https://doi.org/10.1016/j.watres.2024.122760>

Received 15 August 2024; Received in revised form 3 November 2024; Accepted 4 November 2024

Available online 13 November 2024

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cost-effective, and aerobic methane oxidation is one of the simplest ways to remove dissolved methane (Li et al., 2021). With oxygen available, the dissolved methane is expected to be oxidized to carbon dioxide. However, the conventional bubbling aeration applied for oxygen supply may result in the stripping of dissolved methane into the atmosphere before it can be consumed by microorganisms (Brindle and Stephenson, 1996; Casey et al., 2000).

In recent years, membrane-aerated biofilm reactor (MABR) has gained popularity as a relatively new technology for aerobic wastewater treatment. Substrates in MABR have a unique counter-diffusion mode, where oxygen is supplied from the membrane lumen and diffuses into the biofilm layers, and other substrates diffuse from the bulk liquid into the inner side of the biofilm (Casey et al., 1999b). Membrane aeration has an oxygen transfer efficiency (OTE) of up to 100 % (Casey et al., 1999a), typically 30–50 % in current applications (He et al., 2021), while conventional bubbling aeration has limited OTE, ranging between 10 and 15 % (Houweling, 2019). Therefore, implementing MABRs can result in significant energy savings in wastewater treatment. Moreover, the bubbleless membrane aeration prevents the stripping of volatile organic compounds (VOCs) into the atmosphere. This innovative technology has been successfully applied to enhance nitrification (Yamagiwa et al., 1994), organic removal (Pankhania et al., 1994), and degradation of various VOCs (e.g., xylene, ammonia, phenol and chlorophenols) (Brindle and Stephenson, 1996; Casey et al., 1999b; Debus and Wanner, 1992; Terada et al., 2006; Wobus et al., 1995; Woolard and Irvine, 1994). However, very few studies investigated the feasibility of using MABR for dissolved methane removal (Adem et al., 2024; Lu et al., 2024). Recently, Lu et al. (2024) successfully coupled partial nitrification, anammox and anaerobic methane oxidation (n-DAMO) processes in a single MABR, with high-level (>90 %) dissolved methane and nitrogen removal achieved simultaneously. The n-DAMO microorganisms were the dominant contributors to the dissolved methane removal, while aerobic methanotrophs played a minor role. However, a relatively long start-up period (>100 days) was observed due to the slow growth rates of n-DAMO microorganisms. Therefore, aerobic methane oxidation in MABRs could be another alternative for reducing methane emission and might have a shorter start-up period because of the one to two-order higher growth rate of aerobic methanotrophs than n-DAMO microorganisms (Modin et al., 2007; Oswald et al., 2016; Reis et al., 2022;

Zimmermann et al., 2021).

This study aims to experimentally investigate the feasibility of removing dissolved methane aerobically, with oxygen provided from bubbleless gas-delivery membranes. To this end, a lab-scale MABR was established to evaluate dissolved methane removal performance during the long-term operation (over 380 days). Due to the contributions of the anammox process, the performance of nitrogen removal was also evaluated. A series of in situ batch tests were conducted to confirm the species-specific nitrogen and dissolved methane conversion rates. Microbial community structure was determined by 16S rRNA gene amplicon sequencing. The outcome of this study proposes an alternative to remove dissolved methane in anaerobically treated effluent while avoiding its emissions caused by traditional bubbling aeration.

## 2. Materials and methods

### 2.1. Reactor configuration and set up

A lab-scale MABR with a total working volume of 180 mL was established (Fig. 1). The reactor was installed with seven bunches of polypropylene hollow fibre membrane modules and each bundle of membrane comprised 210 fibres (Mitsubishi, Japan), which took up a total volume of 31.1 mL (17.3 mL for fibre materials and 13.8 mL for fibre lumen). The outer diameter and the length of the hollow fibre were 300  $\mu\text{m}$  and 30 cm, respectively, leading to an area/volume ratio of 2308  $\text{m}^2/\text{m}^3$ . One side of the membrane module was connected to an air pump with an airflow meter, which was used to adjust and maintain the air flux. The other side was connected to the atmosphere.

The MABR was fed with synthetic wastewater, which mimicked the anaerobically treated wastewater, containing ammonium and dissolved methane. The ammonium concentration varies from tens of mg N/L to hundreds of mg N/L from sewage to anaerobic digestion liquor. We selected 300 mg N/L ammonium for this proof-of-concept study. In contrast, the dissolved methane concentration is usually 10–20 mg  $\text{CH}_4/\text{L}$ , regardless of the wastewater strength. The influent compositions in different operation phases are summarised in Table 1, with trace elements supplied with the influent according to Liu et al. (2023a). The feed was stored in a 10 L tank and transferred into the reactor by a peristaltic pump (BT300-2J, Longer, China). To maintain the gas

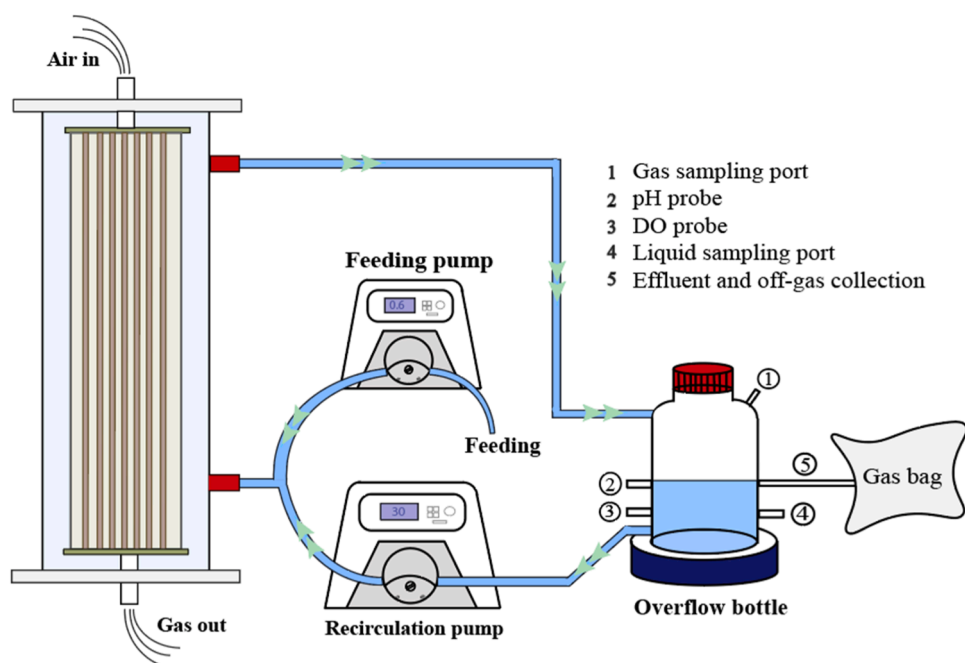


Fig. 1. Schematic diagram of the lab-scale membrane aerated biofilm reactor (MABR).

**Table 1**

Operational strategies and conditions during the long-term experiment.

Phase	Period (days)	Feeding characteristics			DO (mg/L)	Purpose
		HRT (d)	NH <sub>4</sub> <sup>+</sup> -N (mg N/L)	TN loading rate (kg N/m <sup>3</sup> /d)		
I Inoculation	1–59	1.0	300	0.32	0	To achieve a stable nitrogen removal baseline.
	59	–	Manually dose NH <sub>4</sub> HCO <sub>3</sub> stock solution to maintain NH <sub>4</sub> <sup>+</sup> -N at 200 mg N/L.			To attach MOB on the biofilm surface.
II	60–380	1.0	300	0.32	10–15	To achieve simultaneous removal of dissolved methane and nitrogen.

pressure and prevent uncontrolled air leakage into the feed, a 10 L gas bag (Tedlar, Australia) filled with methane gas (95 % CH<sub>4</sub> and 5 % CO<sub>2</sub>, Coregas, Australia) was connected to the feed tank. An overflow bottle (570 mL) was connected to the MABR for sampling, as well as pH (Oakton, Australia) and dissolved oxygen (DO) monitoring (HACH HQ40d, USA). The bulk liquid was recirculated from the bottom to the top by a peristaltic pump (BT300-2J, Longer, China) to ensure that the liquid was completely mixed. The reactor was operated in a temperature-controlled lab (~22 °C) and the pH was generally self-maintained at 7.0 ± 0.5. A 5 L gas bag (Tedlar, Australia) was connected to the overflow bottle for off-gas and effluent collection.

## 2.2. Reactor operation strategies

The long-term experiment consisted of two major phases: Phase I for a nitrogen removal baseline and Phase II for coupling nitrogen removal with aerobic methane oxidation (Table 1). The MABR was initially inoculated with 50 mL biomass from a pilot-scale PN/A reactor treating anaerobic digestion liquor in the Luggage Point Wastewater Treatment Plant (Brisbane, Australia), consisting of ammonia-oxidizing bacteria (AOB) and anammox bacteria. In Phase I (Day 1–59), the reactor was operated under hydraulic retention time (HRT) of 1 d and fed with 295.7 ± 7.6 mg NH<sub>4</sub><sup>+</sup>-N/L to achieve a stable nitrogen removal baseline. Afterwards, the reactor was inoculated with 50 mL biomass with enriched methane-oxidizing bacteria (MOB) (volatile suspended solid, VSS of 1.7 g/L) (Ma et al., 2024). Following the inoculation, the system was operated in batch mode for 1 d to ensure the attachment of the suspended biomass to the biofilm surface. To avoid biomass wash-out, only a stock solution of ammonium bicarbonate (10 g NH<sub>4</sub><sup>+</sup>-N/L) was manually dosed into the reactor and the concentration of ammonium was maintained at ~200 mg NH<sub>4</sub><sup>+</sup>-N/L. Methane gas was injected into the headspace to support the growth of MOB. The system was changed back to continuous mode at HRT of 1 d after the bulk liquid became clear and then switched into Phase II (Day 60–380). From Day 60, dissolved methane was supplied from feeding with ammonium to mimic the anaerobically treated wastewater, with a concentration in the range of 10–15 mg CH<sub>4</sub>/L.

## 2.3. In situ batch tests to determine the microbial activities

Batch tests were carried out at the end of the long-term operation to confirm the active bio-reactions in the established biofilm (Table 2). For Batch tests A, B and C, the reactor was flushed with nitrogen gas for 15 min to ensure the residual dissolved methane was eliminated. Batch test A was designed to investigate the nitrogen removal performance without the influence of dissolved methane, with the initial ammonium concentration set to 50 mg N/L and DO at ~0.1 mg O<sub>2</sub>/L. Batch B was conducted to confirm the activity of nitrite-oxidizing bacteria (NOB), with nitrite spiked at 20 mg N/L and DO controlled at ~0.1 mg O<sub>2</sub>/L. Batch C was designed to investigate the activity of anammox bacteria under anaerobic conditions, with the initial ammonium and nitrite concentrations adjusted to 100 and 20 mg N/L, respectively.

In Batch D and E, the reactor was flushed with methane gas (95 % CH<sub>4</sub> + 5 % CO<sub>2</sub>) for 15 min at the beginning of each test, giving an initial

**Table 2**

Experiment designs for in situ batch tests to determine the microbial activities.

Batch test	O <sub>2</sub> (mg/L)	CH <sub>4</sub> (mg CH <sub>4</sub> /L)	NH <sub>4</sub> <sup>+</sup> (mg N/L)	NO <sub>2</sub> <sup>-</sup> (mg N/L)	NO <sub>3</sub> <sup>-</sup> (mg N/L)	Putative active populations
A	~0.1	0	50	0	0	AOB + NOB + Anammox bacteria
B	~0.1	0	0	20	0	NOB
C	0	0	100	20	0	Anammox bacteria
D	~0.1	~10	0	0	0	MOB
E	~0.1	~10	50	0	0	AOB + NOB + Anammox bacteria + MOB

dissolved methane concentration of about 10 mg CH<sub>4</sub>/L. Batch D was conducted to determine the activity of MOB, with dissolved methane and oxygen available. Differently, the condition of Batch E was the same as the long-term operation, with ammonium, dissolved methane, and oxygen available. Specifically, the reactor was spiked with ammonium at 50 mg N/L and DO was controlled at ~0.1 mg O<sub>2</sub>/L. In each test, liquid samples (4 mL in total) were taken to determine the concentrations of nitrogen species and dissolved methane.

## 2.4. Chemical analysis

The influent and effluent samples (2 mL for each) were taken two or three times a week to determine the concentrations of ammonium, nitrite, and nitrate. The collected liquid samples were filtered through 0.45 µm sterile Millipore filters (Merck) and assayed with a Lachat QuickChem8000 Flow Injection Analyzer (Lachat Instrument, Milwaukee, WI) for nitrogen species determination. Additional influent samples (2 mL) were taken three times a week for dissolved methane measurements. The collected liquid samples were injected into 3 mL vacuum vials and then gas samples (0.1 mL) were taken from the vial headspace when the liquid-gas equilibrium was reached. The collected gas samples were measured with gas chromatography (Agilent GC7890A, Agilent Technologies, Santa Clara, California, USA) and the dissolved methane concentration calculated based on Henry's law (Sander, 2015). The methane concentration in the off-gas was determined by taking gas samples (0.1 mL) from the headspace of the gas bag (Fig. 1) and measuring by gas chromatography. The dissolved methane removal rate and efficiency were calculated according to Lu et al. (2024).

## 2.5. Microbial community analysis

Four biofilm samples were collected on Day 0, 56, 150, and 372 for DNA extraction using FastDNA SPIN Kit for Soil (MP Biomedicals, Santa Ana, CA, USA) according to the manufacturer's instructions. The extracted DNA concentration and quality were examined by NanoDrop 2000 (Thermo Fisher Scientific, Wilmington, DE, USA). Furthermore, the universal primers 926F (5'-AAACTYAAAKGAATTG ACGG-3') and 1392R (5'-ACGGGCGGTGTGTRC-3') were used to amplify the 16S rRNA genes. The amplified 16S rRNA genes were sequenced at the Australian

Centre for Ecogenomics (Brisbane, Australia) and analyzed based on the ACE pyrotag pipeline (Li et al., 2023).

### 3. Results and discussion

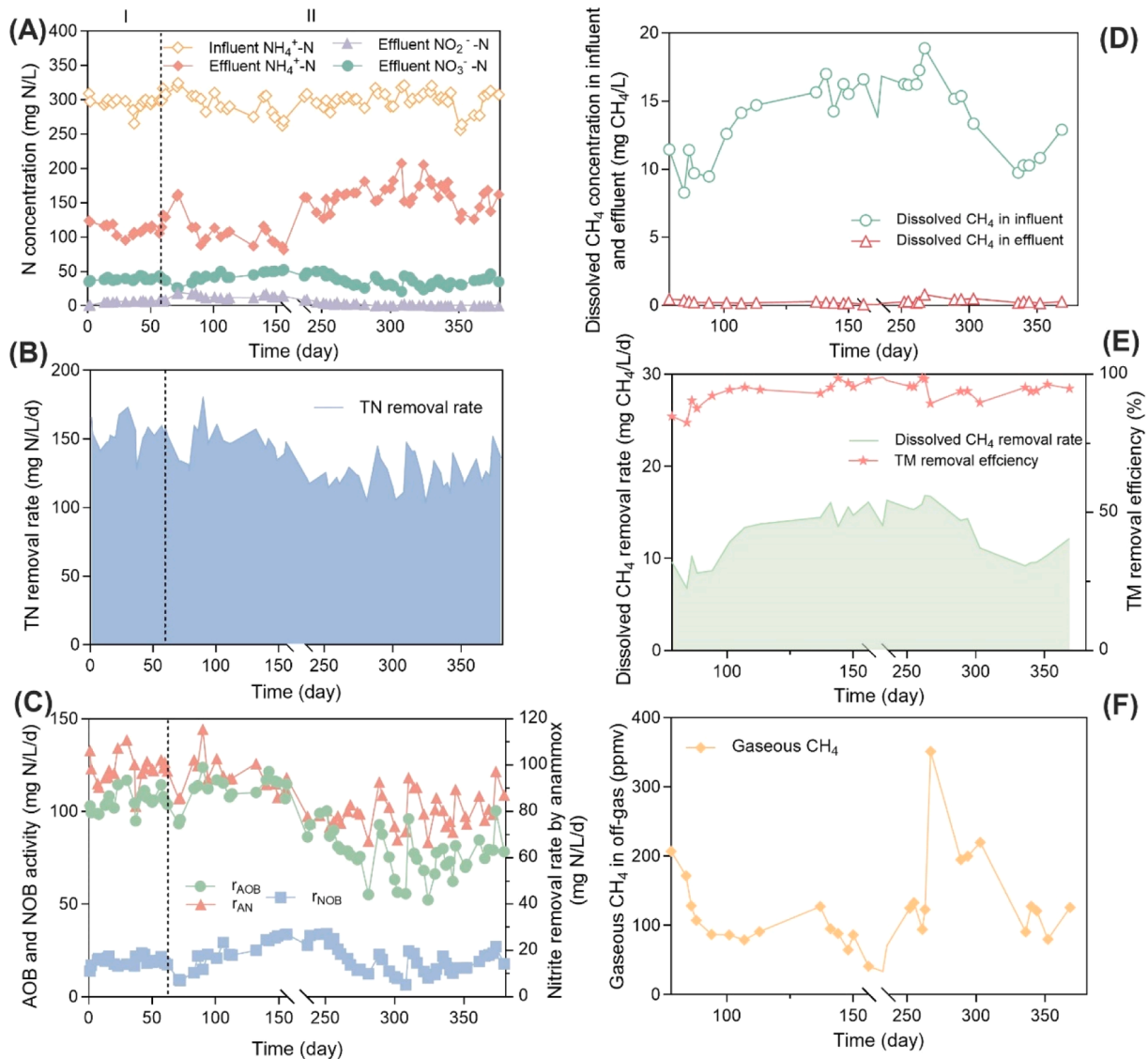
#### 3.1. Nitrogen and dissolved methane removal performance during the long-term operation

##### 3.1.1. Long-term nitrogen removal performance

The MABR was established and operated for 380 days in total, consisting of two major phases (Table 1 and Fig. 2). A baseline of a stable nitrogen removal was established in Phase I (Day 1–59), and the feasibility of coupling aerobic methane oxidation with nitrogen removal was investigated in Phase II (Day 60–380). In Phase I, the reactor was operated under HRT of 1 d and fed with  $295.7 \pm 7.6$  mg N/L ammonium (Fig. 2A). After 60 days of operation, the nitrogen removal performance reached a steady state, with a TN removal rate of  $153.4 \pm 9.9$  mg N/L/d ( $0.28 \pm 0.02$  g N/m<sup>2</sup>/d). (Fig. 2B). Around 60 % of ammonium was converted to nitrite by AOB. The produced nitrite was consumed by NOB

and anammox bacteria, with nitrite consumption rates of  $19.4 \pm 2.5$  and  $98.3 \pm 6.3$  mg N/L/d, respectively (Fig. 2C). The nitrite consumed by NOB was well-sustained below 20 %, indicating the stable NOB suppression in the MABR.

The MABR was inoculated with enriched MOB on Day 59 and operated in batch mode for 1 day for biofilm attachment. Afterwards, the reactor was switched into Phase II and fed with dissolved methane ( $13.9 \pm 3.1$  mg CH<sub>4</sub>/L) and ammonium ( $300.0 \pm 14.9$  mg N/L). The HRT was maintained for 1 day. At the beginning of Phase II (Day 60–80), a slight increase of residual ammonium and nitrite in effluent was observed (Fig. 2A). This phenomenon could be attributed to the oxygen competition between MOB, AOB, and NOB. After the initial incubation period, the effluent ammonium dropped back to  $114.3 \pm 22.8$  mg N/L, with nitrite and nitrate at  $14.2 \pm 3.4$  and  $41.4 \pm 8.5$  mg N/L, respectively. The TN removal rate was stabilized at  $143.9 \pm 17.0$  mg N/L/d ( $0.26 \pm 0.03$  g N/m<sup>2</sup>/d) after a 160-day operation. In situ batch tests were conducted between Day 160 and 230 to determine the impact of the residual ammonium on the nitrogen removal performance (Fig. S1 in Supplementary Information, SI). The results indicated that the activities



**Fig. 2.** Long-term reactor performance of nitrogen and dissolved methane removal. (A): concentrations of various nitrogen species in influent and effluent; (B) the profile of TN removal rate; (C) various species-specific nitrogen removal rates; (D) profiles of dissolved methane concentrations in influent and effluent; (E) total methane removal efficiency (TMRE) and removal rate; (F) gaseous methane concentration in the off-gas.  $r_{\text{AOB}}$ : ammonia oxidation rate by AOB,  $r_{\text{NOB}}$ : nitrite oxidation rate by NOB,  $r_{\text{AN}}$ : nitrite removal rate by anammox bacteria.



of AOB, NOB, and anammox bacteria in the MABR were highly impacted by the concentration of residual ammonium, which played a significant role in NOB suppression. Afterwards, the MABR was further operated for 150 days. In the later stage of Phase II (Day 230–380), the TN removal rate was maintained at  $131.8 \pm 26.0$  mg N/L/d ( $0.24 \pm 0.05$  g N/m<sup>2</sup>/d).

### 3.1.2. Long-term dissolved methane removal performance

Dissolved methane started to be supplied in the feeding from Day 60, with an initial concentration of  $11.5 \pm 2.3$  mg CH<sub>4</sub>/L (Day 60–132) (Fig. 2D). A high-level (~90 %) total methane removal was obtained within a short time (<20 days) and well-sustained (Fig. 2E). Specifically, the dissolved methane in effluent was undetectable (Fig. 2D), while the gaseous methane in the off-gas was determined to be only  $119.9 \pm 46.7$  ppmv (Fig. 2F). Afterwards, the influent dissolved methane was elevated to  $14.4 \pm 3.1$  mg CH<sub>4</sub>/L (Day 133–380). Notably, the total methane removal efficiency (TMRE) stabilized at ~95 %, with negligible dissolved methane in the effluent and only tens of ppmv methane in the collected off-gas. In situ batch tests were conducted between Day 160–230, but there was no negative impact on the subsequent dissolved methane removal. The results conclusively indicate that the MABR can remove dissolved methane effectively.

### 3.2. Determination of active bioreactions by in situ batch tests

Five in situ batch tests were conducted at the end of the long-term operation to determine the active microbial populations and decouple the combined reactions (Fig. 3 and Table S1). Firstly, Batch A was designed to investigate nitrogen removal performance without methane, in which ammonium was supplied with oxygen. DO was controlled at  $0.09 \pm 0.01$  mg O<sub>2</sub>/L, a similar oxygen level applied in the long-term operation. The gradually decreased ammonium with accumulated nitrate in Batch A indicated the active anammox-based nitrogen removal process (Fig. 3A), and the activity of AOB and anammox bacteria was determined to be  $28.4$  mg NH<sub>4</sub><sup>+</sup>-N/L/d and  $20.8$  mg NO<sub>2</sub><sup>-</sup>-N/L/d, respectively (Table S1). The ratio of produced nitrate to TN removed was 0.33, which was higher than the theoretical ratio (0.13) (Strous et al., 1998), indicating the activity of NOB. Batch B was further conducted to determine the maximum activity of NOB, with sufficient nitrite and without other competitors. In Batch B, the nitrite was converted to nitrate (Fig. 3B) and the activity of NOB was calculated to be  $24.6$  mg N/L/d. In Batch C, the maximum activity of anammox bacteria was determined to be  $66.8$  mg N/L/d (Table S1), with unlimited ammonium and nitrite (Fig. 3C).

Methane was supplied in Batch tests D and E. Batch D was designed to investigate the maximum methane removal rate by MOB, and Batch E was conducted to investigate the simultaneous nitrogen removal and aerobic methane oxidation. In Batch D, methane was supplied with oxygen as the sole electron acceptor and an aerobic methane oxidation rate of  $4.5$  mg CH<sub>4</sub>/L/d (equivalent to  $18.1$  mg COD/L/d) was achieved (Table S1). Differently, ammonium was also available in Batch E and a methane removal rate of  $2.5$  mg CH<sub>4</sub>/L/d (equivalent to  $10.1$  mg COD/L/d) was obtained (Table S1). Moreover, the comparison of the NOB activity in Batch A, B and E indicated that NOB could be partially suppressed by the competition with AOB for oxygen and with anammox for nitrite (Table S1).

### 3.3. Microbial community characterization

The shift of the microbial populations during the long-term operation period was elucidated by 16S rRNA gene amplicon sequencing (Fig. 4). Four biomass samples were taken from the MABR on Days 0, 56, 150 and 372. At genus level, “*Candidatus Brocadia*” (anammox bacteria) was detected as the most dominant microorganism, with a relative abundance of 2.1–24.7 % (Fig. 4A), thus being recognized as the major contributor to the nitrogen removal in this MABR. *Denitratisoma* ranked as the second most dominant microorganism, accounting for 4.2–8.8 %,

which has the capability of heterotrophic denitrification to further support nitrogen removal in this ecosystem. It was reported that *Denitratisoma* could live on the carbon compounds excreted by other microorganisms and were previously detected in other anammox-based reactors (Cao et al., 2016; Wang et al., 2017). *Comamonas* and *Comamonadaceae* uncultured were two genera from family *Comamonadaceae*, which accounted for 4.6–6.9 % and 1.0–4.1 % of the microbial community, respectively. *Comamonas* is abundant in biological wastewater treatment systems (Palanisamy et al., 2022) and is recognized to be involved in denitrification (Gumaelius et al., 1996; Patureau et al., 1994; Wang et al., 2004; Wu et al., 2015). Therefore, they might contribute to nitrogen removal in this system to a certain extent. In addition to the capability of nitrogen removal, *Comamonas* was also reported to have biofilm-forming properties (Andersson et al., 2009, 2008; Wu et al., 2015), making it nearly ubiquitous in biofilm systems wastewater treatment systems (Hem et al., 2022). *Rhodanobacter* is considered as another important contributor to denitrification and might play a role in microbial aggregation (Aqeel et al., 2016; Green et al., 2012), which accounted for 0.7–1.6 % of the microbial community. *Terrimonas* and an uncultured genus belonging to the family *Chitinophagaceae* were detected in this biofilm system, with a total relative abundance of 3.5–7.8 %. *Terrimonas* has also been reported as an important contributor to microbial aggregation in the anammox community (Zhao et al., 2019). Collectively, diverse microorganisms capable of nitrogen removal and microbial aggregation co-existed in the established MABR. However, how these microbes interact and metabolize the nitrogen species requires further in-depth investigations.

In addition to these nitrogen removal contributors, there were other flanking partners detected in the system. Two different genera of AOB (*Nitrosomonas* and *Nitrosococcus*) were detected in all samples and co-existed in the system, but with low relative abundance of 0.4–1.5 % and 0.1–1.4 %, respectively. NOB (*Nitrobacter*) were also present (not shown in Fig. 4A due to low abundances) and active in the biofilm (Fig. 2C), with a relative abundance lower than 0.3 %. Meanwhile, MOB including *Methylocystis* and *Methylomonas* were detected with a total relative abundance of 0.3–0.7 % (not displayed in Fig. 4A). *Methylophilus*, an obligate methylophil that uses methanol as the source of carbon and energy (Ginige et al., 2004; Lu et al., 2014), was present in the biofilm samples and determined to be 0.1–0.8 % (Fig. 4A). Notably, one unexpected observation was that “*Candidatus Methylomirabilis*”, was detected in this system, which is well known as the nitrite-dependent anaerobic methane oxidation bacteria (n-DAMO bacteria). An increasing relative abundance from 0.3 to 2.5 % was observed after Day 150. The available methane and nitrite provided a niche for the growth of n-DAMO bacteria under oxygen-limiting conditions. Based on the comparison, this enriched n-DAMO bacteria genus was close to the new species, which was reported to perform both nitrite and nitrate-dependent anaerobic methane oxidation with high tolerance against oxygen (Li et al., 2023; Wu et al., 2024). The presence of n-DAMO bacteria suggested their potential role in removing nitrogen and dissolved methane in the MABR. However, considering that multiple microbial reactions concurrently occurred in the present system, it was impossible to accurately estimate the contribution of the n-DAMO pathway to the overall nitrogen removal. Future studies using isotope tracing would be favourable to elucidate the contribution of each different pathway.

Variations in the relative abundances of known functional microbial populations during the entire experiment were further analyzed and presented in Fig. 4B, including AOB, NOB, MOB and anammox bacteria. Notably, the relative abundance of anammox bacteria dominated over other populations (AOB, NOB and MOB) in all biomass samples. This result indicated that anammox bacteria could be sustained well with limited oxygen level (DO < 0.1 mg/L), potentially because of their lower decay rate than other populations (Mannucci et al., 2020; Munz et al., 2011; Wang et al., 2018). Even though a significantly higher relative abundance of anammox bacteria ( $14.5 \pm 9.7$  %) than AOB ( $1.4 \pm 0.9$  %)

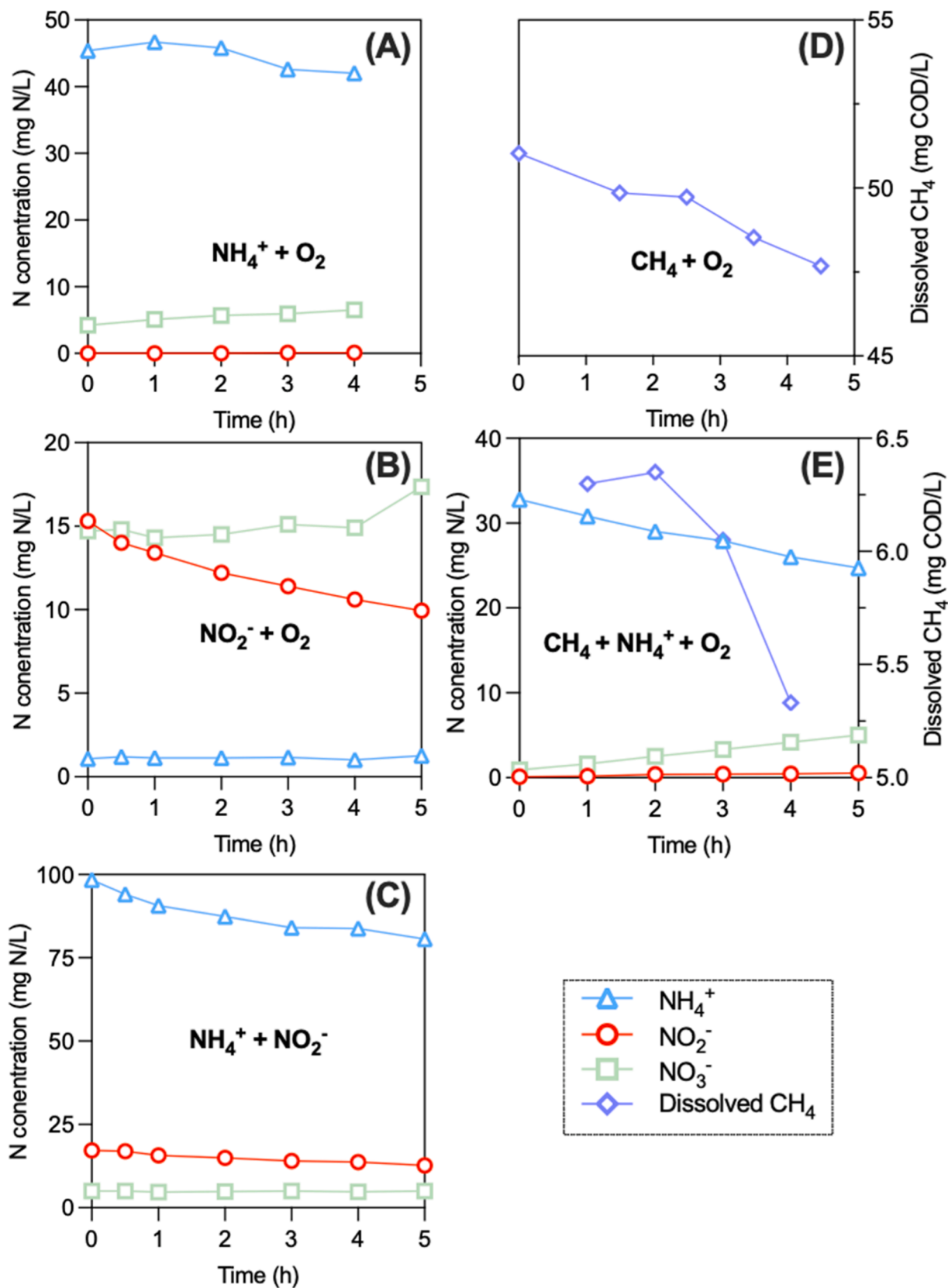
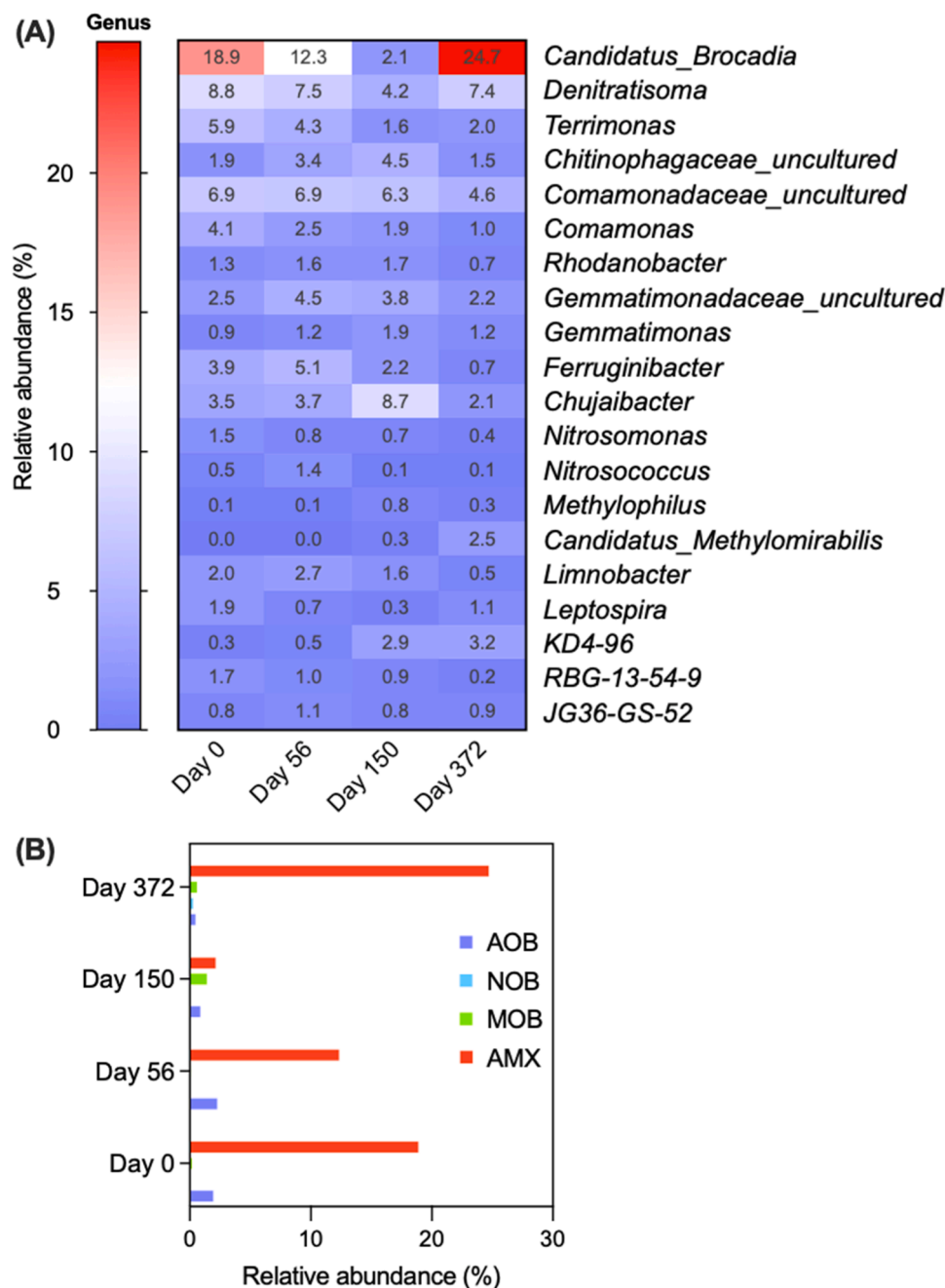


Fig. 3. In situ batch test results under different conditions. (A):  $\text{NH}_4^+ + \text{O}_2$ ; (B):  $\text{NO}_2^- + \text{O}_2$ ; (C):  $\text{NH}_4^+ + \text{NO}_2^-$ ; (D):  $\text{CH}_4 + \text{O}_2$ ; and (E):  $\text{CH}_4 + \text{NH}_4^+ + \text{O}_2$ .



**Fig. 4.** Shift of microbial community structure during the experiment. (A): Heat map displaying genera with the top 20 most relative abundance in at least two samples; (B): relative abundances of the major functional microbial groups, including AOB, NOB, MOB, and anammox bacteria (AMX).

was observed, there was no apparent difference between their activities (Fig. 2C). It has been reported that the relative abundance cannot be linked to microbial activities directly (Ishii et al., 2013; Speth et al., 2016). Furthermore, a high-level (>90 %) dissolved methane removal was achieved in the MABR, with a relative abundance of MOB lower than 1.4 %.

### 3.4. Engineering implications

To transform WWTPs from large energy consumers to energy exporters, anaerobic technologies have been implemented to recover the bioenergy from wastewater. However, anaerobic processes might not be environmentally friendly because of dissolved methane stripping from anaerobically treated effluent, which is a technical hurdle for the

widespread application of anaerobic treatment (Liu et al., 2014). The biological conversion of methane into carbon dioxide is one of the simplest ways to remove dissolved methane (Li et al., 2021). However, a large proportion of dissolved methane would be stripped out during aeration when conventional bubbling aeration was applied, contributing to the carbon footprint of WWTPs (Chen et al., 2015; Daelman et al., 2012).

In recent decades, there has been increasing interest in MABR technology which uses gas-delivery membranes to supply oxygen. The minimized stripping of VOCs through bubbleless aeration, the high oxygen transfer rates and the capability to degrade contaminants through multiple redox gradient zones, ensure the feasibility of MABRs for treating industry wastewater (Quan et al., 2018) and sulfur recovery (Cai et al., 2017; Sun et al., 2017). Furthermore, MABRs are also

reported for the degradation of xenobiotics, including phenolic compounds (Hu et al., 2022; Tian et al., 2020; Wang et al., 2021), and fluorinated organics (Heffernan et al., 2009; Misiak et al., 2011). Specifically, the counter-diffusion of the oxygen and other substrates in MABRs results in the formation of stratified biofilm. Thereby, MABRs are considered the best-suited reactor configuration for simultaneous nitrification and denitrification (Ravishankar et al., 2022) and autotrophic nitrogen removal via anammox (Chen and Zhou, 2022; Li et al., 2016), showing a good performance and significant energy saving. The  $\text{N}_2\text{O}$  production and emissions in MABRs were significantly less than that in conventional co-diffusion biofilm reactors (e.g. granular and moving bed biofilm reactors), because of the adjacent locations of the  $\text{N}_2\text{O}$  production and reduction zones (Kinh et al., 2017a, 2017b). However, only a few studies reported the application of MABRs for dissolved methane removal (Adem et al., 2024; Lu et al., 2024). The gas-delivery membrane aeration can sustain the dissolved methane in the bulk liquid, rather than being stripped out by conventional bubbling aeration. In this study, the anammox-based nitrogen removal was coupled with aerobic methane oxidation in a one-stage MABR. The separation and counter-diffusion of gaseous and liquid fluxes prevent the stripping of the dissolved methane, which can be directly consumed by MOB. The results of this work demonstrated that the dissolved methane could be removed efficiently by the MABR, with a removal efficiency of over 90 %.

A nitrogen removal rate of  $\sim 150 \text{ mg N/L/d}$  ( $0.27 \text{ g N/m}^2/\text{d}$ ) was simultaneously achieved through the combined anammox process, with a mild-strength feeding of  $300 \text{ mg NH}_4^+-\text{N/L}$  and a TN loading rate of  $300 \text{ mg N/L/d}$ . This proof-of-concept study demonstrated the feasibility of the simultaneous removal of dissolved methane and nitrogen by coupling aerobic methane oxidation with the shortcut nitrogen removal process. However, the TN removal rate achieved in the MABR was limited to target high-strength wastewater treatment but sufficient for mainstream application. Therefore, future investigations of this technology in treating domestic mainstream wastewater could be considered. Furthermore, it is important to acknowledge that the hydrodynamic conditions in a larger-scale MABR are expected to differ from those observed in the small reactor (180 mL) used in this study, particularly for mass transfer and biofilm development. Therefore, future studies should also focus on scaling-up experiments to evaluate the practical feasibility and potential for large-scale applications. In addition, further demonstration using real wastewater is necessary, which is the premise of future scaling-up. The presence of organic carbon in wastewater will facilitate the growth of heterotrophs which may compete with anammox bacteria. The temperature variations of real wastewater could be another challenge, considering that the low temperature in the winter would limit the growth and activity of anammox bacteria (Dosta et al., 2008). Therefore, appropriate operation strategies should be carefully designed for a successful demonstration under real conditions.

### 3.5. Comparing methane removal through aerobic or anaerobic methane oxidation in MABRs

Anaerobic methane oxidation can be directly coupled to denitrification. Two specific microbial groups, nitrite/nitrate-dependent anaerobic methane oxidation bacteria/archaea (n-DAMO bacteria and archaea), were discovered to utilize methane as the carbon source to support nitrite/nitrate reduction (Haroon et al., 2013; Raghoebarsing et al., 2006). Coupling n-DAMO processes with PN/A in MABR achieved high-level (>90 %) dissolved methane and nitrogen removal in parallel (Lu et al., 2024), with a dissolved methane and nitrogen removal rate of  $30 \text{ mg CH}_4/\text{L/d}$  ( $0.05 \text{ g CH}_4/\text{m}^2/\text{d}$ ) and  $70 \text{ mg N/L/d}$  ( $0.12 \text{ g N/m}^2/\text{d}$ ), respectively.

However, the slow growth rates of n-DAMO bacteria and archaea ( $\mu_{\text{max,DB}} = 0.043 \text{ d}^{-1}$  and  $\mu_{\text{max,DA}} = 0.036 \text{ d}^{-1}$ ) result in a long start-up period (>100 days) (Chen et al., 2014), which limits its applicability for

engineering purposes. MOB are more competitive because of their high growth rate ( $\mu_{\text{max,MOB}} = 0.16\text{--}5.5 \text{ d}^{-1}$ ) (Modin et al., 2007; Oswald et al., 2016; Reis et al., 2022; Zimmermann et al., 2021). Aerobic methane oxidation showed a shorter start-up time (<20 days) in the present study. Specifically, dissolved methane could be removed immediately once MOB were inoculated (Fig. 2D and E), while anaerobic methane oxidation took around 60 days to show marked contribution after the inoculation of n-DAMO microorganisms (Lu et al., 2024). Moreover, n-DAMO microorganisms were recognized as sensitive to oxygen stress, even at a trace level (Guerrero Cruz et al., 2018; Kampman et al., 2018; Luesken et al., 2012). The functional genes from the denitrification pathway were severely downregulated with the addition of only 2 % oxygen, resulting in reduced nitrogen removal performance. In contrast, MOB can efficiently remove dissolved methane with no oxygen sensitivity and higher methane affinity (Guerrero Cruz et al., 2018). Therefore, coupling PN/A with aerobic methane oxidation provides another alternative for the simultaneous removal of dissolved methane and nitrogen in future implementations.

Collectively, both aerobic and anaerobic methane oxidations have their advantages. Aerobic methanotrophs have a higher growth rate inducing shorter start-up periods, a higher methane affinity and no sensitivity to oxygen as n-DAMO populations. In contrast, anaerobic methane oxidation (e.g., n-DAMO) can remove nitrogen using dissolved methane, despite the relatively slower growth rate. During the long-term operation, one unexpected observation was the presence of n-DAMO bacteria in the system, even with aerobic methanotrophs as the inoculum without any n-DAMO. Therefore, the present system coupled the advantages of both aerobic and anaerobic methane oxidations. This process removes nitrogen via anammox and n-DAMO bacteria while mitigating dissolved methane via both aerobic and anaerobic methane oxidation.

## 4. Conclusions

In this study, aerobic methane oxidation was applied in a lab-scale MABR to remove dissolved methane from synthetic anaerobically treated wastewater. The major conclusions and outcomes include:

- A high level (>90 %) of dissolved methane removal and simultaneous nitrogen removal ( $\sim 150 \text{ mg N/L/d}$ ) were achieved in the one-stage MABR.
- Aerobic methanotrophs were mainly responsible for the dissolved methane removal, while the nitrogen removal was attributed to joint contribution of AOB and anammox bacteria “*Ca. Brocadia*”.
- Coupling nitrogen removal with aerobic methane oxidation showed a shorter start-up period (<20 days) than anaerobic methane oxidation (>100 days).
- Gas-delivery membrane aeration prevents the stripping of dissolved methane and enables a high-level dissolved methane removal efficiency.

## CRedit authorship contribution statement

**Yan Lu:** Writing – original draft, Validation, Investigation, Formal analysis. **Tao Liu:** Writing – review & editing, Supervision, Conceptualization. **Hui Wang:** Funding acquisition. **Lukun Zuo:** Funding acquisition. **Shihu Hu:** Supervision. **Zhiguo Yuan:** Supervision. **Wayne Bagg:** Writing – review & editing. **Jianhua Guo:** Writing – review & editing, Supervision, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. An author of this submission, Jianhua Guo, serves as an editor of Water Research. The manuscript has been



handled and reviewed independently by other editors of the journal, with Jianhua Guo recusing themselves from any decisions related to the evaluation and acceptance of this work.

## Acknowledgements

This work is supported by the Australian Research Council Linkage Project (LP220200963). Tao Liu is a recipient of the Hong Kong Research Grants Council's Early Career Scheme (No. 25238324). We thank Ms. Jianguang Li, Dr Andrea Hernandez Vallejo and Mr. Nigel Dawson for their assistance with FIA measurements.

## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.watres.2024.122760](https://doi.org/10.1016/j.watres.2024.122760).

## Data availability

Data will be made available on request.

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