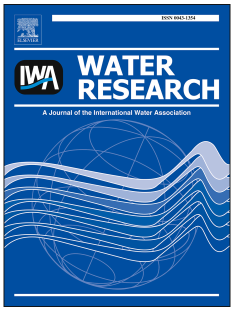

Nitrate Reduction by Denitrifying Anaerobic Methane Oxidizing Microorganisms can reach a practically useful rate

Chen Cai, Shihu Hu, Jianhua Guo, Ying Shi, Guo-Jun Xie, Prof. Zhiguo Yuan

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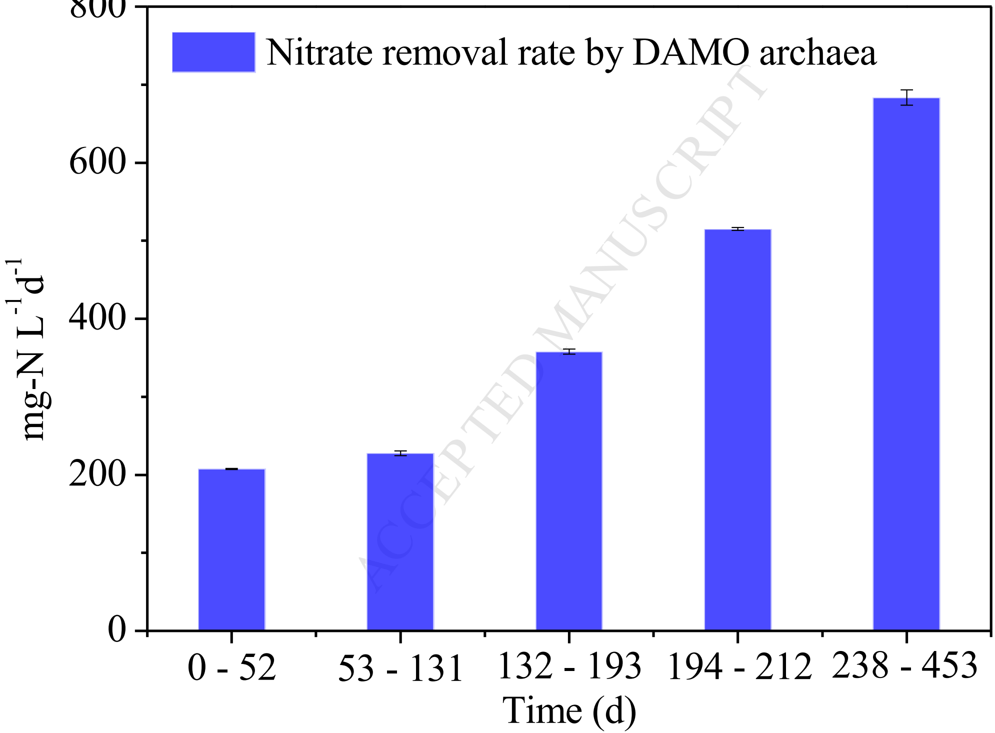
$$NO_3^- + 2/8CH_4 \longrightarrow NO_2^- + 2/8CO_2 + 4/8H_2O$$



Nitrate Reduction by Denitrifying Anaerobic Methane Oxidizing Microorganisms can reach a practically useful rate

Chen Cai, Shihu Hu, Jianhua Guo, Ying Shi, Guo-Jun Xie, Zhiguo Yuan*

Advanced Water Management Centre, The University of Queensland, St Lucia, QLD 4072, Australia

*Corresponding author

Prof. Zhiguo Yuan

Tel.: +61 (0)7 3365 4373

fax: +61 (0)7 3365 4726.

Email: z.yuan@awmc.uq.edu.au

Abstract

Methane in biogas has been proposed to be an electron donor to facilitate complete nitrogen removal using denitrifying anaerobic methane oxidizing (DAMO) microorganisms in an anammox reactor, by reducing the nitrate produced. However, the slow growth and the low activity of DAMO microorganisms cast a serious doubt about the practical usefulness of such a process. In this study, a previously established lab-scale membrane biofilm reactor (MBfR), with biofilms consisting of a coculture of DAMO and anammox microorganisms, was operated to answer if the DAMO reactors can achieve a nitrate reduction rate that can potentially be applied for wastewater treatment. Through progressively increasing nitrate and ammonium loading rates to the reactor, a nitrate removal rate of 684 ± 10 mg-N $L^{-1}d^{-1}$ was achieved after 453 days of operation. This rate is, to our knowledge, by far the highest reported for DAMO reactors, and far exceeds what is predicted to be required for nitrate removal in a sidestream (5.6 to 135 mg-N $L^{-1}d^{-1}$) or mainstream anammox reactor (3.2 to 124 mg-N $L^{-1}d^{-1}$). Mass balance analysis showed that the nitrite produced by nitrate reduction was jointly reduced by anammox bacteria at a rate of 354 ± 3 mg-N $L^{-1}d^{-1}$, accompanied by an ammonium removal rate of 268 ± 2 mg-N $L^{-1}d^{-1}$, and DAMO bacteria at a rate of 330 ± 9 mg-N $L^{-1}d^{-1}$. This study shows that the nitrate reduction rate achieved by the DAMO process can be high enough for removing nitrate produced by anammox process, which would enable complete nitrogen removal from wastewater.

Key words: anaerobic methane oxidation; membrane biofilm reactor; *Candidatus* Methanoperedens nitroreducens; nitrate reduction rate; nitrogen removal; anammox

1. Introduction

Throughout most of the twentieth century, both denitrifying anaerobic methane oxidation (DAMO) and anaerobic ammonium oxidation (anammox) processes were thought to be "impossible" (Strous and Jetten, 2004). The discovery of DAMO and anammox microorganisms has not only dramatically changed the understanding of the global carbon and nitrogen cycles, but also opened some perspectives to achieve high levels of nitrogen removal with a minimized carbon footprint during wastewater treatment (Guo et al., 2013).

Anammox is an autotrophic process and is able to convert ammonium to nitrogen gas anaerobically with nitrite as the sole electron acceptor (van de Graaf et al., 1996, 1997; Kuenen, 2008):

$$NO_2^- + 1/1.32NH_4^+ \rightarrow 1.02/1.32N_2 + 0.26/1.32NO_3^- \qquad (1)$$

The identification of the responsible chemolithoautotrophic bacteria, i.e. anammox bacteria (Strous et al., 1999), stimulated the appreciation of their applied and ecological significance. Moreover, the anammox process is an economically attractive and environmentally friendly alternative to current wastewater treatment, enabling a high-level bioenergy recovery and resulting in less sludge production, oxygen supply decrease and N_2O emissions reduction (Kartal et al., 2010a; Kartal et al., 2010b; Hu et al., 2013). The partial nitrification-anammox process, has to date attracted considerable attention for its application to treat various types of wastewaters (e.g. anaerobic digestion liquor, landfill leachate and industrial wastewaters) (Hippen et al., 2001; van der Star et al., 2007; Joss et al., 2009; Abma et al., 2010). Both the one-stage processes, e.g. CANON (Completely Autotrophic Nitrogen removal Over Nitrite) (Jetten et al., 2001), OLAND (Oxygen-Limited Autotrophic Nitrification-Denitrification) (Kuai and Verstraete, 1998), and the two-stage process known as the

SHARON (Single reactor system for High activity Ammonium Removal Over Nitrite)-anammox process (van Dongen et al., 2001) have been installed and operated in full-scale. For example, stable sidestream treatment of anaerobic sludge digestion liquor with an ammonium concentration higher than 500 mg-N L^{-1} has been widely applied in full-scale wastewater treatment plants (van Hulle et al., 2010). More significantly, there is a growing realization about expanding the sidestream anammox technology towards mainstream applications (Jetten et al., 1997; Kartal et al., 2010a). Despite the challenges caused by the low nitrogen concentration (<100 mg-N L^{-1}) and low, ambient temperature associated with mainstream wastewater (Hendrickx et al., 2012), several studies showed that nitrogen removal could be achieved with the anammox process from mainstream wastewater (Lotti et al., 2014b; Lotti et al., 2015).

In addition to the relatively long start-up time caused by the anammox bacteria's long doubling time (11-20 days) (Strous et al., 1998; Jetten et al., 2009), which is being addressed through growing large quantities of seeding cultures, the anammox process presents some other limitations. According to Equation 1, even with an optimal ammonium to nitrite molar ratio of 1:1.32 in the feed, the anammox process can only remove 89% of the total nitrogen theoretically, with 11% of the nitrogen converted to nitrate. The nitrogen removal efficiency reported in literatures was normally around 70%, since the effluent from the partial nitritation reactor cannot ensure the ideal ratio of 1:1.32 (van Hulle et al., 2010; Lotti et al., 2014a).

The discovery of the DAMO process, in which methane is oxidized anaerobically to provide electrons for denitrification (Raghoebarsing et al., 2006; Hu et al., 2009; Ettwig et al., 2010; Haroon et al., 2013), provides new opportunities to achieve nitrogen removal from wastewater by utilizing methane as the electron donor under anaerobic conditions (Luesken et al., 2011; Shi et al., 2013). Several recent studies confirmed the presence of microorganisms able to anaerobically oxidize methane with nitrite or nitrate as the electron acceptor (Ettwig et al., 2010; Haroon et al., 2013). Ettwig et al. (2010) identified a novel bacterium, '*Candidatus* Methylomirabilis oxyfera', which is able to reduce nitrite to nitrogen gas with methane as the electron donor, while Haroon et al. (2013) discovered a novel archaeon, '*Candidatus* Methanoperedens nitroreducens', which is capable of converting nitrate to nitrite using methane as the electron donor. These microorganisms are collectively called DAMO microorganisms. The reactions mediated by DAMO archaea and DAMO bacteria are summarized as Equations 2 and 3, respectively.

$$NO_3^- + 2/8CH_4 \rightarrow NO_2^- + 2/8CO_2 + 4/8H_2O \qquad (2)$$

$$NO_2^- + 3/8CH_4 + H^+ \rightarrow 1/2N_2 + 3/8CO_2 + 10/8H_2O \qquad (3)$$

The discovery of Equation 2 provides a possibility of achieving complete nitrogen removal in an anammox reactor by supplying biogas (containing methane) as an electron donor to DAMO organisms. Several recent studies have indeed demonstrated that anammox and DAMO organisms can grow in a single reactor fed with ammonium, nitrate/nitrite and methane (Luesken et al., 2011; Haroon et al., 2013; Ding et al., 2014; Hu et al., 2015). Two bioreactors seeded with the same inocula (DAMO archaea, DAMO bacteria and anammox bacteria) were operated by feeding nitrate and nitrite as electron acceptors, respectively. Although fed with different electron acceptors, DAMO archaea dominated both reactors with anammox bacteria as a flanking partner. However, DAMO bacteria disappeared when the reactors reached stable state (Hu et al., 2015). In another study, ammonium was supplied to a culture dominated by DAMO bacteria in a sequencing batch reactor (SBR). After 161 days of enrichment, a coculture dominated by DAMO bacteria and anammox bacteria was established. The nitrite removal rate of the coculture was 100 mgN $L^{-1}d^{-1}$, and 33% of which was contributed by DAMO bacteria (Luesken et al., 2011). These two studies indicated that DAMO organisms and anammox bacteria could build a relationship with each other and they were capable of consuming nitrate/nitrite and ammonium simultaneously. In spite of the feasible coexistence of DAMO organisms and anammox bacteria, the nitrogen removal rates (NRRs) of the cocultures in these two studies were only 25 (Hu et al., 2015) and 135 mg-N $L^{-1}d^{-1}$ (Luesken et al., 2011), respectively. Particularly, the nitrate/nitrite reduction rates of DAMO organisms were only 13 and 33 mg-N $L^{-1}d^{-1}$, respectively, which were orders of magnitude lower than that required for practical applications (Luesken et al., 2011; Hu et al., 2015).

Recognizing the potential of nitrogen removal via a partnership between anammox and DAMO organisms, Shi et al (2013) investigated the possibility of achieving a higher NRR with the use of a membrane biofilm reactor (MBfR). In this system, hollow fiber membranes were used to supply methane and also to provide a surface for the growth of the slow-growing DAMO and anammox organisms. Nitrate and ammonium were periodically directly fed to the liquid phase. Simultaneous nitrate and ammonium removal was achieved in this reactor at a rate of 190 mg-N $L^{-1}d^{-1}$ and 60 mg-N $L^{-1}d^{-1}$, respectively. Isotopic studies revealed that nitrogen removal was achieved through a partnership of DAMO archaea, DAMO bacteria and anammox bacteria. While the rates are an order of magnitude higher than those obtained in the previous studies with suspended culture (Luesken et al., 2011; Kampman et al., 2012; Kampman et al., 2014; Hu et al., 2015), these rates, without further improvement, would not enable the practical application of the combined DAMO and anammox processes for nitrogen removal.

The aim of this work is to reveal if the DAMO organisms can catalyze nitrate reduction at a rate that is practically useful for wastewater treatment under optimal conditions despite their low biomass-specific activity. To this end, we progressively increased the nitrate and ammonium loading rates to the MBfR reported in Shi et al. (2013) and subsequently operated the MBfR as a continuous reactor rather than a SBR. The nitrate and ammonium removal rates of the MBfR were measured to evaluate the reactor performance under different operational conditions. The data were then analyzed with a mass balance model to estimate the rates of all relevant reactions (Equations 1-3 listed above).

2. Methods

2.1 MBfR set-up

The setup of the MBfR system in this work is shown in Figure 1. One bundle of hollow-fiber membrane, consisting of 900 polyacrynlonitrile hollow fibers with a total surface area of 1 m^2, was fixed inside a polysulphone tube as the membrane module (AIP-2013, Pall, Japan). The length of each hollow fiber is 552 mm with an inside diameter of 0.8 mm. The fiber is made of composite materials. The outer and inner layers are made up of macroporous material. Between these two layers is a dense porous layer. The total volume of the membrane module is 1150 mL, comprising a working volume of 450 mL for liquid flow and biofilm growth, a volume of 300 mL inside the hollow fibers for gas delivery, and a volume of 400 ml for fiber material occupation.

The bottom end of the hollow fibers was linked to a gas cylinder, and the feeding gas was forced to penetrate through the wall of hollow fibers by sealing the top end of the hollow fibers. The gas pressure of interior hollow fibers was monitored by a gas-pressure gauge (Ross Brown, Australia) and manually adjusted by the regulator connected to the gas cylinder.

A 2.4 L glass bottle was used to store fresh medium containing nitrate and ammonium. To prevent air leaking into the vessel, a 3 L gas bag containing nitrogen gas was connected to the bottle. The medium was transported to the bulk liquid through a feeding pump. The medium and bulk liquid was quickly mixed and recirculated by a peristaltic pump (Masterflex, USA) from the bottom of the reactor to the top.

A 330 mL overflow bottle with 180 mL headspace was set up to keep the liquid volume of the MBfR at the same level. The liquid inside the bottle was mixed by a magnetic stirrer (Labtek, Australia) at 200 rpm and the pH of which was being monitored by a pH meter (Oakton, Australia). Liquid samples were collected through the liquid sampling ports on the bottle to evaluate the performance of the MBfR. A water seal bottle was connected to the overflow bottle to release nitrogen gas and CO_2 produced in the MBfR and residual methane, also prevented air from getting into the system. The liquid volume of the overflow bottle was not considered during HRT calculation, since there was no biological activity in the bottle.

2.2 Gas and medium

The gas mix supplied to the reactor was composed of 90% CH_4, 5% CO_2 and 5% N_2 (Coregas, Australia). The fresh medium components (per liter) were as follows: KH_2PO_4, 0.075 to 0.11 g; $CaCl_2 \cdot 2H_2O$, 0.3 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; $NaNO_3$, 3.643 to 9.107 g; NH_4Cl, 1.146 to 1.529 g; acidic trace element solution, 0.5 mL, alkaline trace element solution, 0.2 mL (Ettwig et al., 2009).

2.3 MBfR operation

The MBfR was operated for about 453 days at 22 ± 2°C. The pH of MBfR was maintained at 7-8 by manually dosing 1 M HCl solution everyday. Two stages, namely SBR stage (Day 0-212) and continuous-feeding stage (Day 238-453), were involved in the operation.

In the SBR stage, a 24 hr cycle consisted of 5 min of 150 mL medium supply (recirculation pump stopped running during this period and 150 mL effluent was discharged at the same time) and 1435 min of biological reaction as described previously (Shi et al., 2013), which resulted in a hydraulic retention time (HRT) of 3 days. At the initial time of the SBR stage (Day 0-52), the concentrations of nitrate and ammonium in influent were 600 mg-N L^{-1} and 300 mg-N L^{-1}, respectively. With the decrease of nitrate and ammonium concentrations in the effluent, the nitrate and ammonium concentrations in the influent were periodically increased. Since the nitrate removal rate increased faster than the ammonium removal rate, the influent nitrate and ammonium concentrations were elevated to 1500 mg-N L^{-1} and 400 mg-N L^{-1} between days 197 and 212, respectively. With the improvement of NRR, continuous-feeding was applied in the second stage (Day 238-453) to avoid the fluctuation of nitrate and ammonium concentrations in the reactor. The influent (nitrate: 1000 mg-N L^{-1}; ammonium: 400 mg-N L^{-1}) feeding rate was controlled at 300 mL d^{-1}, which led to a decreased HRT of 1.5 days. The concentrations of nitrate and ammonium in the influent was maintained at 1000 and 400 mg-N L^{-1}, respectively, resulting in a constant NLR of 933 mg-N $L^{-1}d^{-1}$. The gas pressure of inner hollow fibers was changed from 1.3 to 1.6 atm in this stage.

2.4 Chemical and microbial analysis

Liquid samples of MBfR were taken regularly to determine the concentrations of NH_4^+-N, NO_2^--N and NO_3^--N. The concentrations of nitrogenous compounds in the influent and effluent were measured by a Lachat QuickChem8000 Flow Injection Analyzer (Lachat Instrument, Milwaukee, WI) (Hu et al., 2009). Volatile suspended solids (VSS) were determined in the effluent to quantify the biomass loss. Fluorescence *in situ* hybridization (FISH) was conducted on Day 453 as described previously (Shi et al., 2013).

2.5 Biological reaction rates determination

The NRR of the MBfR system was determined by the net ammonium oxidation rate (rNH_4^+) and nitrate reduction rate (rNO_3^-). FISH test indicated that DAMO archaea (50%), DAMO bacteria (20%) and anammox bacteria (20%) jointly dominated the microbial community in the biofilm. Based on the theoretical yields of the DAMO and anammox organisms (Chen et al., 2014), biodegradability of yielded biomass (Lee and Rittmann, 2000) and the amount of biomass washed out within effluent, the organic matter available for denitrification in the MBfR was calculated as only 0.019 g-VSS $L^{-1}d^{-1}$ at the final steady stage. Its contribution to the total denitrification rate was estimated to be below 2%, which is negligible. Therefore, three biological reactions, namely nitrate reduction by DAMO archaea (r1), nitrite reduction by DAMO bacteria (r2) and ammonium oxidation by anammox bacteria (r3), were considered as the dominating nitrogen conversion reactions in the MBfR system.

Based on the Equations 1-3, the nitrogen conversion rates r1, r2 and r3 can be shown as follows:

$$r3 = rNH_4^+ \quad (4)$$

$$r1 = rNO_3^- + 0.26\ rNH_4^+ \quad (5)$$

$$r2 = rNO_3^- + 0.26\ rNH_4^+ - 1/1.32\ rNH_4^+ \quad (6)$$

3. Results

3.1 Performance of the MBfR

The MBfR (as shown in Figure 1) was operated in two stages over a period of 453 days. The nitrate, nitrite and ammonium concentrations in the influent and effluent were measured regularly (Figure 2a). These measurements, along with the hydraulic loading rates, were used to calculate the total nitrogen loading rates (NLRs), the nitrate and ammonium removal rates and the total NRRs, with the results shown in Figure 2b.

In the SBR stage (Day 0 - 212), the nitrate removal rate, ammonium removal rate and total NRR remained relatively stable at 183 ± 12 mg-N $L^{-1}d^{-1}$, 45 ± 5 mg-N $L^{-1}d^{-1}$ and 228 ± 14 mg-N $L^{-1}d^{-1}$, respectively, prior to the first change of NLR on Day 53. By Day 40, the effluent nitrate concentration became negligible, indicating complete nitrate removal. On Day 53, the nitrate concentration in the influent was increased from 600 mg-N L^{-1} to 700 mg-N L^{-1}. Both the nitrate and ammonium removal rates decreased slightly following the change; however, both recovered in the following 35 days, which triggered further increase in the influent nitrate concentration to 1000 mg-N L^{-1} on Day 137. Indeed, an exponential increase of the nitrate, ammonium and total nitrogen removal rates occurred during days of 150 to 212, with the progressive increase in the NLR. The nitrate removal rate reached 485 mg-N $L^{-1}d^{-1}$ at the end of this period, while NLR reached 633 mg-N $L^{-1}d^{-1}$ (1500 mg-NO_3^--N L^{-1}, 400 mg-NH_4^+-N L^{-1}). No nitrite accumulation was observed during the entire phase, with the nitrite concentration in the effluent mostly below 1.0 mg-N L^{-1} (Figure 2a).

In the continuous-feeding mode during days of 238 to 453, the HRT was shortened to 1.5 days from 3 days with the influent nitrate and ammonium concentrations at 1000 and 400 mg-N L^{-1}, respectively, to further increase the NLR to 933 mg-N $L^{-1}d^{-1}$. Unfortunately, accidental pressure losses from the gas cylinder due to faulty connecting tubing occurred on Day 238, 312 and 380 (shown by arrows in Figure 2b), which caused sharp drops in the reactor performance in all cases. Biomass was visible in the effluent after the accidents, indicating part of the biomass was detached from the hollow fibers. It took approximately two months in each case for the reactor performance to fully recover, causing relatively large variations in the performance. However, the rates returned to similar values after each recovery. During Day 433 to 453, during which the reactor performance was stable, the average nitrate, ammonium and total nitrogen removal rates were 614 ± 10 mg-N $L^{-1}d^{-1}$, 268 ± 2 mg-N $L^{-1}d^{-1}$, and 882 ± 11 mg-N $L^{-1}d^{-1}$, respectively, representing approximately 92%, 100% and 95% of the respective loading rates. These values are similar to those in other periods when the reactor fully recovered. Like in the SBR phase, no nitrite accumulation was observed in this phase, with the nitrite concentration in the effluent mostly below 1.0 mg-N L^{-1} (Figure 2a).

3.2 Rates of key reactions (Equations 1-3)

FISH measurement revealed that DAMO archaea (50%), DAMO bacteria (20%) and anammox bacteria (20%) jointly dominated the microbial community in the biofilm, which meant that other microorganisms formed a small part of the microbial population. The calculation of the contribution of heterotrophic denitrification to nitrate and nitrite removal (less than 2%) corroborated the microbial data. Both these results suggested that the three reactions (Equation 1-3) were the dominant bioprocesses in this reactor. Hence, the above-presented (apparent) nitrate and ammonium removal rates and the absence of nitrite accumulation enable the calculation of the rates of Equations 1-3 with Equations 4-6. This subsequently enables the calculation of the nitrate reduction rate of DAMO archaea (catalyzing Equation 2) and the nitrite reduction rate by anammox (catalyzing Equation 1) and DAMO bacteria (catalyzing Equation 3). These rates during the continuous operation phase are shown in Figure 3. The average ‘normal’ (i.e. with data in the disturbed periods removed) nitrate removal rate by DAMO achaea was 684 ± 10 mg-N $L^{-1}d^{-1}$, while the average 'normal' nitrite removal rate by DAMO bacteria and anammox bacteria was 330 ± 9 and 354 ± 3 mg-N $L^{-1}d^{-1}$, respectively. The nitrate removal rate by DAMO archaea was approximately 11% higher than the apparent nitrate removal rate (614 ± 10 mg-N $L^{-1}d^{-1}$). This may be because that, in addition to removing nitrate in the feed, DAMO archaea also removed nitrate produced by the anammox reaction. The nitrite production rate by DAMO archaea should be equivalent to its nitrate removal rate (i.e. 684 ± 10 mg-N $L^{-1}d^{-1}$). DAMO bacteria and anammox bacteria are estimated to remove approximately 48% and 52%, respectively, of the nitrite produced.

4. Discussion

Although methane-supported biological nitrate/nitrite removal from wastewater has been investigated in several lab-scale studies, the removal rates achieved were always too low to be practically applicable. This has become a major bottleneck for applying this technology in practice (Kampman et al., 2012; Shi et al., 2013; Kampman et al., 2014). Table 1 summarizes the DAMO-supported nitrate and nitrite reduction rates reported in literature to date, in comparison with the anammox process. The nitrate reduction rate achieved in this study was 684 mg-N $L^{-1}d^{-1}$, which is 2.3 times higher than that obtained in Shi et al. (2013) and 7.2 – 135.8 times higher than other rates (Table 1). To the best of our knowledge, this is the highest nitrate reduction rate by DAMO organisms to date, indicating that DAMO microorganisms have a great capacity of removing nitrate.

The NRRs of the anammox process, either in one-stage or in two-stage systems, in sidestream wastewater treatment, were normally between 50 and 1200 mg-N $L^{-1}d^{-1}$ (Hu et al., 2013). Thus the nitrate production rates of sidestream anammox process ranged from 5.6 to 135 mg-N $L^{-1}d^{-1}$ (i.e. 11% of the anammox NRR). Similarly, the

NRRs in mainstream anammox process in recent studies were generally between 28 and 1100 mg-N $L^{-1}d^{-1}$ (Regmi et al., 2014), which led to the nitrate production rates from 3.2 to 124 mg-N $L^{-1}d^{-1}$. It means that complete nitrogen removal can only be obtained when the nitrate reduction rate reaches 135 mg-N $L^{-1}d^{-1}$ or higher. The nitrate reduction rate in this study is much higher than what required as calculated, demonstrating that the DAMO process is capable of removing nitrate completely in anammox systems. In theory, complete nitrogen removal can still be achieved when the NRR of the anammox process is up to 6104 mg-N $L^{-1}d^{-1}$, which is much higher than what acquired in most lab-scale or full-scale nitrogen removal systems involving anammox.

This high nitrate reduction rate in the MBfR could be attributed to several factors. Firstly, biomass retention was recognized as a vital factor for good nitrogen removal by DAMO and anammox microorganisms due to their slow growth rate (Tang et al., 2011; Kampman et al., 2012; Shi et al., 2013). The uncoupling between SRT and HRT in the MBfR can efficiently prevent the microorganisms from being washed out of the system (Syron and Casey, 2008), which is particularly important for the proliferation of slow-growing microorganisms such as DAMO and anammox microorganisms. In the proposed MBfR, hollow-fiber membrane was used as a carrier for microorganism attachment. Biofilm was visible on the out-layer of membrane and biomass in the effluent was hardly visible during operation. The biomass loss rate in the effluent was only 0.006 g-VSS $L^{-1}d^{-1}$ in the final steady stage, indicating superior biomass retention in the MBfR system. Secondly, elevated nitrate loading rate might have stimulated the growth of DAMO archaea, which was supported by the visible increase of biofilm thickness. Also the percentage of DAMO archaea increased to 50% of the microbial population compared to 20-30% in Shi et al., (2013). Therefore the increase of nitrate removal rate in the reactor may be mainly due to the increase of DAMO archaea biomass. Thirdly, the continuous-feeding mode applied during the operation and the decrease of HRT from 3 days to 1.5 days accelerated liquid discharge from the MBfR. It was speculated that accumulation of inhibiting products might confine the activity of DAMO organisms (Ettwig et al., 2008; Kampman et al., 2012). Decrease of the HRT may have helped wash out the potential inhibitors when the microbial activity was at a high level.

Although a high nitrate reduction rate was obtained in this study, the rate decreased severely after pressure losses in the methane gas delivery line. The losses of pressure inside the hollow fibers caused biomass detachment from the biofilm (biomass was observed in the effluent). Although the MBfR was quickly re-pressurized when losses of pressure were detected, the nitrogen removal rate kept decreasing for a couple of weeks. This could be attributed to the fact that detached biomass was trapped in the dense fibers and was only completely washed out in a few weeks. The suspended biomass was still active, which may explain the slow drop of reactor performance. The performance could only be completely recovered after around two months every time, revealing that the reactor is sensitive to the failure of biomass retention. Thus, further research is needed to improve the robustness of the MBfR system.

It should be noted that, with the aim of revealing the potentially achievable nitrate reduction rate by DAMO organisms, nitrate was used in the feed along with ammonium. The fact that no nitrite accumulation was observed during the study suggests that the activities of anammox and DAMO bacteria were limited by the activity of DAMO archaea. In practice, the feed to an anammox reactor comprises mainly nitrite and ammonium (preferably at a ratio of 1.32:1). Hence, the microbial community in the biofilm would be significantly different from that in our reactor.

However, the DAMO archaea population is expected to develop, due to the simultaneous presence of nitrate and methane, and the absence of electron donors supporting the development of ordinary nitrate reducers. Our study suggests that DAMO archaea population can be retained in the biofilm, catalyzing the removal of nitrate at a satisfactory rate and therefore facilitating a high-level of nitrogen removal that is otherwise difficult to achieve in an anammox reactor. In our system, DAMO bacteria were present removing 48% of the nitrite. We hypothesize the abundance of DAMO bacteria may be related to the limited supply of ammonium (relative to the availability of nitrite) in our system. This may not be the case in a MBfR fed with nitrite and ammonium at a proper ratio. Based on the known kinetics of anammox bacteria and DAMO bacteria, anammox bacteria are expected to have a competitive advantage over DAMO bacteria (Luesken et al., 2011; Hu et al., 2015). However, a detailed MBfR study with ammonium and nitrite in the feed is required to get a full understanding of the population dynamics and reactor performance.

5. Conclusion

This study evaluated the feasibility of improving the nitrate reduction rate for complete nitrogen removal in a MBfR system. The main conclusions are drawn as follows:

- A high level of nitrate reduction rate (684 ± 10 mg-N $L^{-1}d^{-1}$) can be achieved by DAMO archaea, which is practically useful for both mainstream and sidestream nitrogen removal.
- Complete nitrogen removal is possible by integrating the DAMO and anammox processes, with methane as the sole electron donor enabling nitrate removal.
- A membrane biofilm reactor is a suitable technology for integrating the anammox and DAMO processes.

- Both the nitrate reduction rate and the whole membrane biofilm reactor performance can be elevated by increasing the nitrogen loading rate and applying a continuous-feeding mode.

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Table 1 Comparison of the DAMO nitrate and nitrite reduction rates and anammox nitrite reduction rate reported to date

Process and reference	Configuration (Types of aggregates)	Temperature (°C)	Microbial composition	Nitrate removal rate[a] (mg-N $L^{-1}d^{-1}$)	Nitrite removal rate (mg-N $L^{-1}d^{-1}$)
Anammox-related processes (Hu et al., 2013)	(Biofilm/suspended sludge/granular/hybrid)	22 - 37	Ammonium oxidizing bacteria; anammox bacteria	-	28 – 683[b]
Synergetic DAMO and anammox processes (Luesken et al., 2011)	SBR (Suspended sludge)	30	Anammox bacteria; DAMO bacteria	-	77 for anammox bacteria; 33 for DAMO bacteria
DAMO process (Kampman et al., 2012)	SBR (Suspended sludge)	30	DAMO bacteria	-	38
DAMO process (Kampman et al., 2014)	Membrane reactor (Suspended sludge and biofilm)	20	DAMO bacteria	-	36
Synergetic DAMO and anammox processes (Haroon et al., 2013)	SBR (Suspended sludge)	22	Anammox bacteria; DAMO archaea	13	13 for anammox bacteria[b]
Synergetic DAMO and anammox processes (nitrate-fed reactor) (Hu et al., 2015)	SBR (Suspended sludge)	35	Anammox bacteria; DAMO archaea	16	16 for anammox bacteria[b]
Synergetic DAMO and anammox processes (nitrite-fed reactor) (Hu et al., 2015)	SBR (Suspended sludge)	35	Anammox bacteria; DAMO archaea	5	25 for anammox bacteria[b]
Synergetic DAMO and anammox processes (Ding et al., 2014)	SBR (Suspended sludge)	35	Anammox bacteria; DAMO bacteria; DAMO archaea	83	8 for DAMO bacteria[c]; 75 for anammox bacteria[b]
Synergetic DAMO and anammox processes (Shi et al., 2013)	MBfR (biofilm)	22	Anammox bacteria; DAMO bacteria; DAMO archaea	206	126 for DAMO bacteria[c]; 80 for anammox bacteria[b]
Synergetic DAMO and anammox processes (this study)	MBfR (biofilm)	22	Anammox bacteria; DAMO bacteria; DAMO archaea	684	330 for DAMO bacteria[c]; 354 for anammox bacteria[b]

a nitrate removal rate of DAMO archaea was calculated by Equation 5.
b nitrite removal rate of anammox bacteria was calculated by Equation 1.
c nitrite removal rate of DAMO bacteria was calculated by Equation 6.

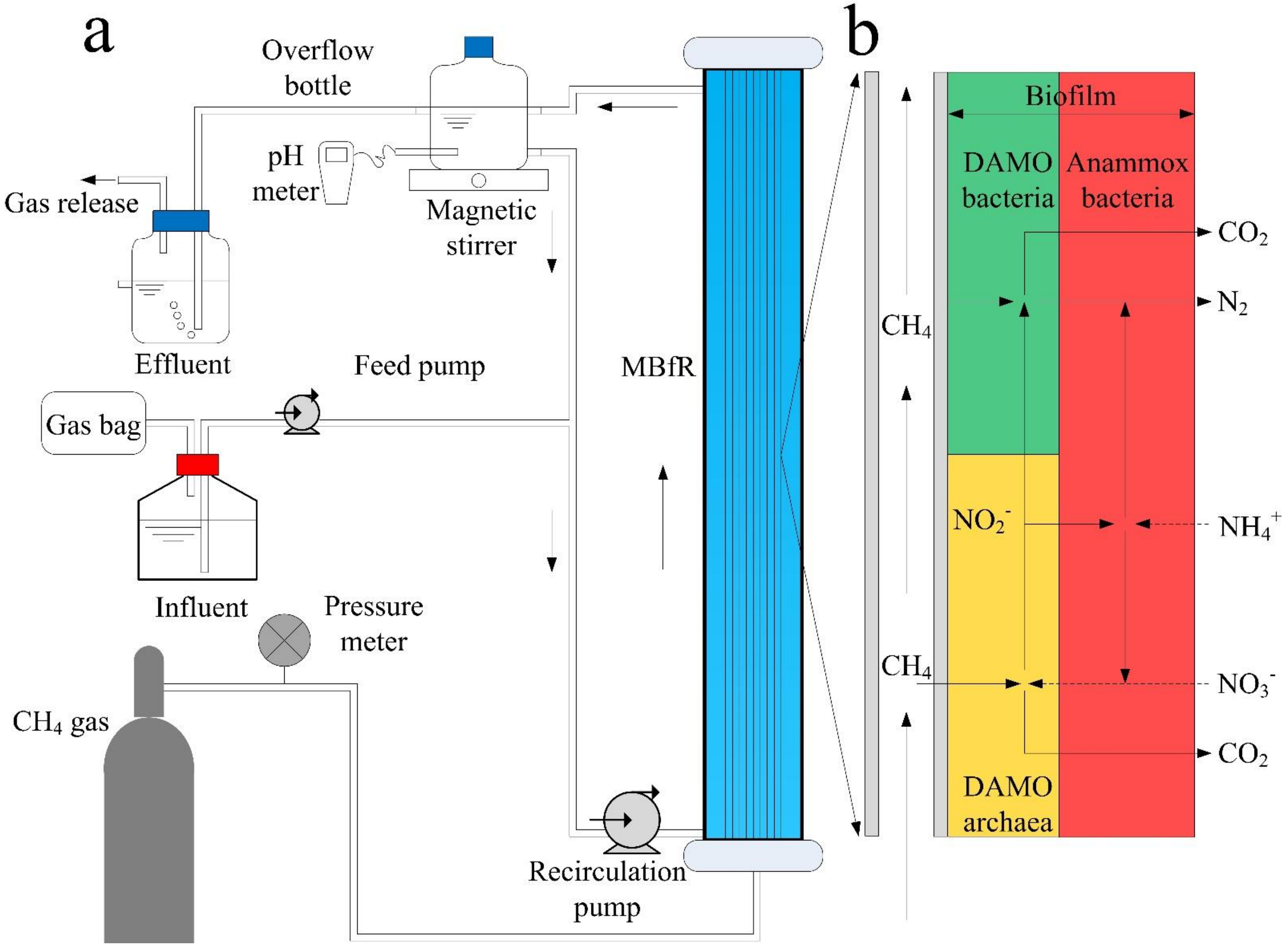

Figure 1. (a) The MBfR setup, and (b) the hypothesized in-biofilm reactions (Shi et al., 2013).

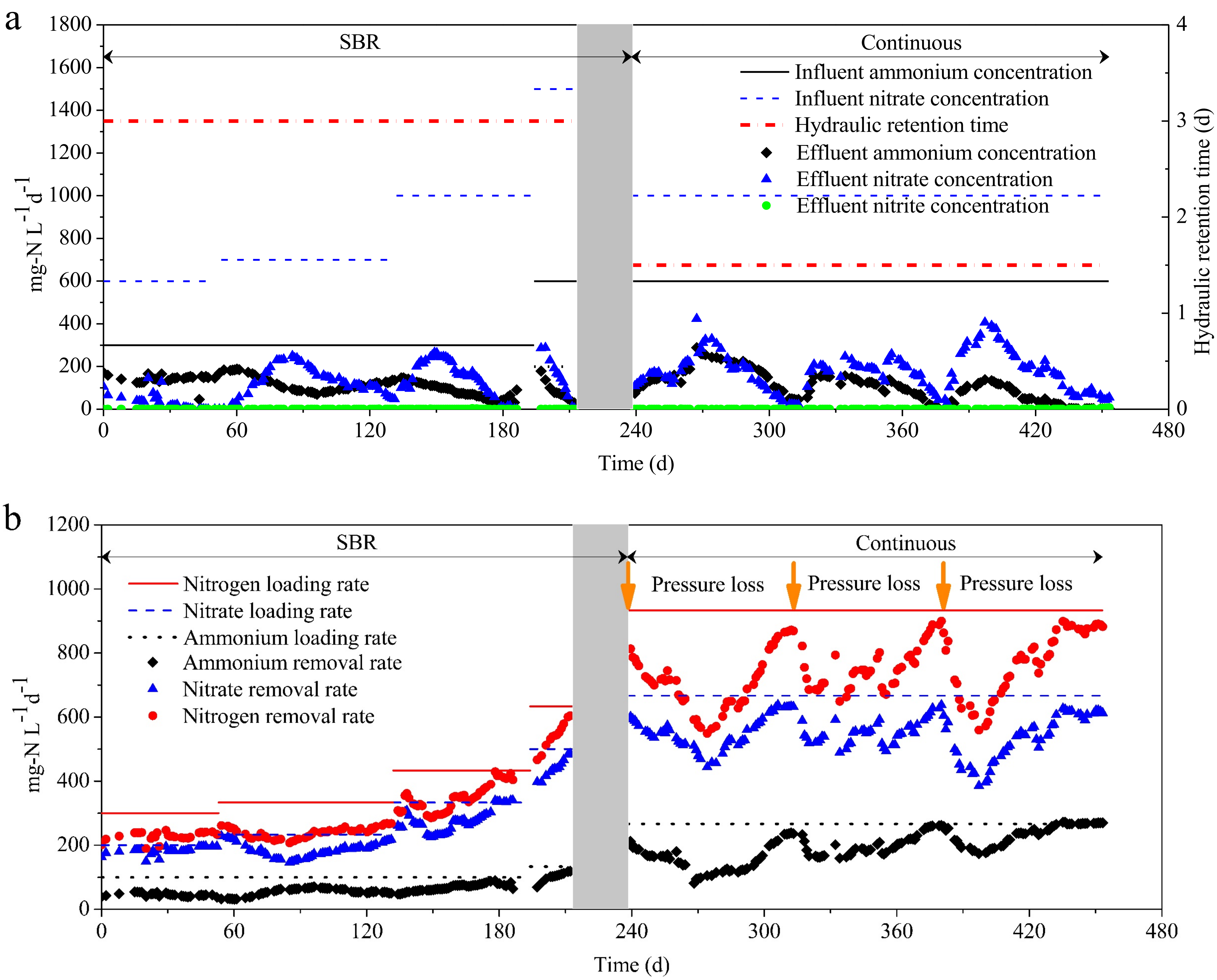

Figure 2 (a) Ammonium and nitrate concentrations in the influent and effluent, and the hydraulic retention time, and (b) the total nitrogen loading rates, and the ammonium, nitrate and total nitrogen removal rates, during 453 days of operation. Arrows on Day 238, 312 and 380 indicated pressure losses in the hollow fibers, leading to reverse permeation of bulk liquid to the interior space of the hollow fibers. Grey box was the transitional period between SBR and continuous mode, which operated with the same conditions as applied in the SBR stage but additional ammonium and nitrate were added to guarantee adequate substrates.

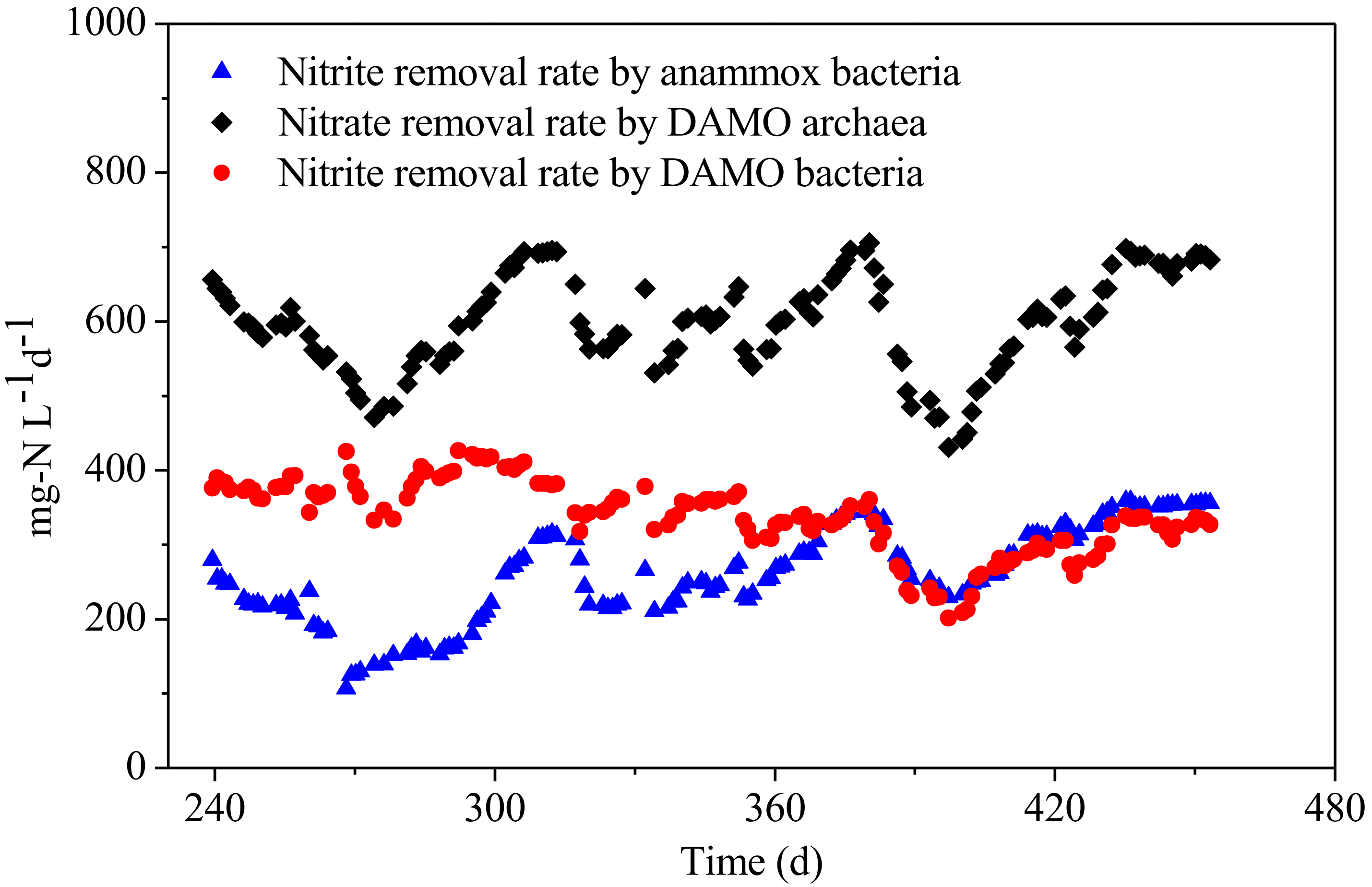

Figure 3 The estimated nitrate removal rate by DAMO archaea, nitrite removal rate by DAMO bacteria and nitrite removal rate by anammox bacteria in the continuous-feeding stage

Highlights

- A coculture of DAMO archaea, DAMO bacteria and anammox bacteria was enriched.
- High nitrate reduction rate was obtained by DAMO archaea.
- The achieved nitrate reduction rate is practically useful for wastewater treatment.
- Biogas is a potential electron donor for nitrogen removal from wastewater.
- A membrane biofilm reactor is a suitable technology for anammox and DAMO processes.