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# Effect of methane partial pressure on the performance of a membrane biofilm reactor coupling methane-dependent denitrification and anammox

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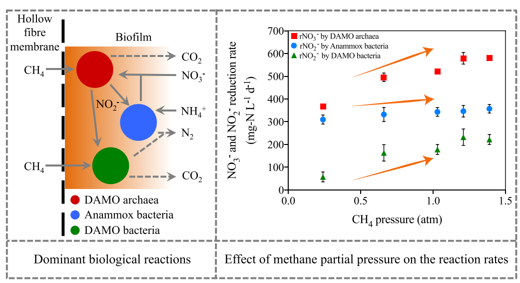
## HIGHLIGHTS

- Activities of DAMO microorganisms can be regulated via methane partial pressure.
- Methane partial pressure is a manipulated variable to control MBfR performance.
- The methane-based MBfR has a high methane utilization efficiency.
- Microbial stratification cannot be formed without substrate gradients.
- Model can predict MBfR performance at different methane partial pressures.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Complete nitrogen removal has recently been demonstrated by integrating anaerobic ammonium oxidation (anammox) and denitrifying anaerobic methane oxidation (DAMO) processes. In this work, the effect of methane partial pressure on the performance of a membrane biofilm reactor (MBfR) consisting of DAMO and anammox microorganisms was evaluated. The activities of DAMO archaea and DAMO bacteria in the biofilm increased significantly with increased methane partial pressure, from 367 $\pm$ 9 and 58 $\pm$ 22 mg-N L$^{-1}$d$^{-1}$ to 580 $\pm$ 12 and 222 $\pm$ 22 mg-N L$^{-1}$d$^{-1}$, respectively, while the activity of anammox bacteria only increased slightly, when the methane partial pressure was elevated from 0.24 to 1.39 atm in the short-term batch tests. The results were supported by a long-term (seven weeks) continuous test, when the methane partial pressure was dropped from 1.39 to 0.78 atm. The methane utilization efficiency was always above 96% during both short-term and long-term tests. Taken together, nitrogen removal rate (especially the nitrate reduction rate by DAMO archaea) and methane utilization efficiency could be maintained at high levels in a broad range of methane partial pressure (0.24–1.39 atm in this study). In addition, a previously established DAMO/anammox biofilm model was used to analyze the experimental data. The observed impacts of methane partial pressure on biofilm activity were well explained by the modeling results. These results suggest that methane partial pressure can potentially be used as a manipulated variable to control reaction rates, ultimately to maintain high nitrogen removal efficiency, according to nitrogen loading rate.



## 1. Introduction

Nitrogen removal from wastewater is important for preventing aquatic eutrophication. As an energy-efficient and cost-effective process, anaerobic ammonium oxidation (anammox) has been

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implemented for full-scale ammonium rich wastewater treatment by integrating with partial nitritation either in one stage or two stages (Hu et al., 2013; Lackner et al., 2014). By using nitrite as sole electron acceptor, anammox bacteria are able to oxidize ammonium to nitrogen gas in the absence of oxygen (Eq. (1)) (Strous et al., 1998). However, several inherent drawbacks hamper efficient nitrogen removal by anammox (Cai et al., 2015). First, anammox converts 11% of the total nitrogen to nitrate, which cannot be further removed by itself (Eq. (1)). Second, the process requires a nitrite to ammonium molar ratio of 1.32 to 1 in the feed, which could not always be maintained steadily in practical operation. These limitations usually result in relatively high nitrogen concentrations in effluent which is non-dischargeable and needs further treatment (Lackner et al., 2014).

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

To polish nitrogen removal efficiency, external electron donors are often required for residual nitrate/nitrite removal (Cherchi et al., 2009). As a cheap and easily available electron donor, methane is considered as an appropriate option for nitrogen removal (Modin et al., 2007). Recently, microorganisms capable of catalyzing anaerobic oxidation of methane with nitrate or nitrite as terminal electron acceptor have been discovered (Ettwig et al., 2010; Haroon et al., 2013; Raghoebarsing et al., 2006). These reactions are referred to as the denitrifying anaerobic methane oxidation (DAMO) process, which is mediated by an archaeon, *Candidatus*'*Methanoperedens nitroreducens*' (DAMO archaea), using nitrate as the electron acceptor (Eq. (2)) (Haroon et al., 2013) and a bacterium, *Candidatus* '*Methylomirabilis oxyfera*' (DAMO bacteria), using nitrite as the electron acceptor (Eq. (3)) (Ettwig et al., 2010), respectively.

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

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

The DAMO process provides a new option for nitrogen removal in a more cost-effective way because methane-containing biogas is readily available in many wastewater treatment plants (WWTP) (Modin et al., 2007). Conversion of methane to $CO_2$ could further potentially mitigate global climate change as methane is a much more potent greenhouse gas than $CO_2$ (IPCC, 2013). Through coupling DAMO and anammox in one configuration, nitrate, either introduced to the system or produced via anammox, could be reduced to nitrite by DAMO archaea, which is crucial to attain complete nitrogen removal. Nitrite can be further removed by either anammox bacteria or DAMO bacteria. The growth of DAMO bacteria enables residual nitrite removal in the system, delivering flexibility of nitrite to ammonium ratio provided (Xie et al., 2017). As a result, complete nitrogen removal is expected to be achievable when linking this integrated DAMO/anammox process to partial nitritation.

The feasibility of coexistence of DAMO and anammox microorganisms has been verified in several previous studies (Ding et al., 2014; Haroon et al., 2013; Hu et al., 2015; Luesken et al., 2011; Zhu et al., 2011). For instance, a coculture of DAMO archaea and anammox bacteria has been enriched by supplying methane, ammonium and nitrate in a sequencing batch reactor (SBR), which achieved nitrate and ammonium removal rates of 13 and 12 mg-N $L^{-1}d^{-1}$, respectively (Hu et al., 2015). Cocultures of DAMO bacteria and anammox bacteria have been enriched from both DAMO bacteria dominating culture (Luesken et al., 2011) and anammox granules (Zhu et al., 2011) feeding with methane, ammonium and nitrite. The nitrite removal rates by these cocultures could reach >100 mg-N $L^{-1}d^{-1}$, although nitrate produced from anammox bacteria could not be removed (Luesken et al., 2011; Zhu et al., 2011).

Membrane technology has been widely adopted for industrial and medical applications, such as reverse osmosis, gas separation, electrodialysis and controlled drug delivery (Baker, 2004). In water sector, anaerobic membrane bioreactor has been applied to wastewater treatment (Wang et al., 2017). Towards complete nitrogen removal in wastewater, a combined technology was developed through coupling membrane biofilm reactor (MBfR) and DAMO/anammox processes (Shi et al., 2013). The key advantages of the MBfR are high biomass retention by biofilm development and efficient methane delivery (Shi et al., 2013), which has been demonstrated to be critical for enhanced nutrient removal (Cai et al., 2015; Shi et al., 2013; Sunil Kumar et al., 2007; Tomar et al., 2015). These features enabled much higher nitrogen and carbon conversion rates compared to the use of suspended cultures in previous studies (Haroon et al., 2013; Hu et al., 2015). Biofilm jointly dominated by DAMO archaea, DAMO bacteria and anammox bacteria has been established after two years of operation, with apparent ammonium and nitrate removal rates of 60 and 190 mg-N $L^{-1}d^{-1}$, respectively (Shi et al., 2013). Through changing the operation mode from SBR to continuous-feeding and successive elevating influent nitrogen loading, substantially improved ammonium and nitrate removal rates of 268 and 684 mg-N $L^{-1}d^{-1}$ were obtained, respectively (Cai et al., 2015). These two proof-of-concept studies demonstrated that simultaneous ammonium, nitrite and nitrate removal can be achieved by synergic DAMO and anammox, and the nitrate removal rate can be high enough for practical applications (Cai et al., 2015; Shi et al., 2013). Indeed, the concept has been applied for treatment of both domestic wastewater (mainstream) and anaerobic digestion liquor (sidestream), resulting in near-complete nitrogen removal using synthetic effluent from partial nitritation (Xie et al., 2017, 2018).

The pressure of the feeding gas in an MBfR has previously been shown to play a key role in reactor performance (Martin and Nerenberg, 2012). A good example is the hydrogen-based MBfR, which has been studied for the removal of various oxidized contaminants with hydrogen as the electron donor (Rittmann, 2006). In Lee and Rittmann (2002), the performance of a hydrogen-based MBfR was assessed with three criteria, i.e. nitrate removal efficiency, hydrogen utilization efficiency, and low nitrite accumulation. Hydrogen partial pressure was found to greatly affect the nitrate removal rate and hydrogen utilization efficiency (Lee and Rittmann, 2002). Under the same nitrate loading rate, increasing hydrogen partial pressure resulted in enhanced nitrate removal and decreased effluent nitrite concentration, but led to increased hydrogen concentration in the effluent thus reduced the hydrogen utilization efficiency. By decreasing hydrogen partial pressure to the MBfR (i.e. to make hydrogen a limiting substrate), a satisfactory nitrate removal rate could still be achieved with relatively low dissolved hydrogen and nitrite concentrations in the effluent.

On the basis of the results above (Lee and Rittmann, 2002), it is hypothesized that methane partial pressure should be an important parameter of a methane-based MBfR and may have a profound effect on its performance. This represents a significant opportunity for reactor operation. As the influent nitrogen loading rate may fluctuate in WWTP (Laureni et al., 2016), methane partial pressure could be used as a variable for maintaining the performance of an MBfR by regulating methane partial pressure. Furthermore, nitrogen removal in a methane-based MBfR is catalyzed by a range of functionally diverse organisms including DAMO archaea, DAMO bacteria and anammox bacteria, working in synergy (Shi et al., 2013). The responses of these microorganisms in terms of the respective nitrate/nitrite reduction rates to the change of methane partial pressure are worth investigating.

In this study, the detailed effects of methane partial pressure on the performance of a methane-based MBfR and on the dominant reactions were evaluated through both experimental and modeling studies. Specifically, the dependency of the activities of the three microorganisms mediating the dominant reactions in the biofilm, i.e. nitrate reduction by DAMO archaea, nitrite reduction by DAMO bacteria and anammox bacteria, on methane partial pressure was first determined in short-term batch tests. These effects were further monitored in a prolonged period following an almost 50% reduction in methane partial pressure.