Applications of methane-based membrane biofilm reactor for oxidized pollutants removal from wastewater: a short review

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Abstract. Methane-based membrane biofilm reactor (CH₄-MBfR) is an emerging wastewater treatment technology based on gas-permeable membranes for oxidized pollutants (e.g., NO₃⁻) abatement. This paper reviews the operation principle of CH₄-MBfR, the involved pathways in the aerobic/anaerobic methane oxidation coupled to denitrification (AMO/ANMO-D) process, as well as the recent key advances of CH₄-MBfR for environmental applications.

1. Introduction
Methane is an important anthropogenic greenhouse gas, with a global warming potential 21 times greater than that of CO₂. The global atmospheric methane emissions are as high as 450-600 Tg per year[1], and are still growing at a rate of 1.0%~1.2% per year[2]. It has been estimated that when methane is utilized as the sole carbon source in a denitrification system, the process cost of denitrifying anaerobic methane oxidation (DAMO) is 15 times lower than methanol supported heterotrophic denitrification[3]. In this regard, methane recycling holds its environmental significance and economic benefit. However, the physichochemical properties of methane, involving flammable, explosive, extremely unstable and low solubility in water, are limiting its large-scale application in sewage disposal facilities. The membrane biofilm reactor (MBfR) is an emerging water treatment technology on the basis of gas-transferring membranes, on which the gaseous electron donors, arising from the intramembrane, are consumed by the attached biofilm to reduce the pollutants (electron acceptor)[4]. By adopting the bubbleless aeration strategy, the MBfR is capable of maximizing the removal rate of contaminants while avoiding the gas transmission risk and solubility limitation. Attributed to the high reducibility, low-cost and widely available nature of electron donor, CH₄-MBfR has gained increasing attention in the area of oxidized pollutants (especially NO₃⁻) elimination from polluted waters[5]. In this paper, we will introduce the operation principle and research process of CH₄-MBfR in terms of oxidized pollutants removal.

2. Operation Principle of CH₄-MBfR
The operation principle of CH₄-MBfR is depicted in Figure 1. Methane diffuses from the hollow fiber membranes to the biofilm in a bubbleless manner, and the functional bacteria in the biofilm can utilize methane as carbon source and electron donor to reduce the oxidized pollutants deriving from the bulk liquid, e.g., NO₃⁻/NO₂⁻, ClO₄⁻ and Cr (VI)[6]. After that, the resultant reductive products like N₂, Cl⁻ and Cr (III) are able to transfer from the biofilm to the bulk liquid[6]. The reaction equations and free energies for diverse oxidized pollutants removal with methane as electron donor are described in Table
1. Among these oxidized pollutants, NO$_3^-$ has drawn broad attention of research community because of its ubiquitous presence in a majority of surface and groundwater. Previous references have shown that the reported maximal NO$_3^-$ removal flux is around 1 g N/m$^2$ per day in CH$_4$-MBfRs, and according to the results of modeling analysis, the currently available hollow fiber membranes were able to support a NO$_3^-$ removal flux as high as 1.7 g N/m$^2$ per day[7].

![Figure 1. Operation principle of CH$_4$-MBfR.](image)

Table 1. Reaction equations and free energies for reduction of diverse oxidized pollutants with methane as electron donor[6].

| Acceptor     | Reaction equation                                                                 | $\triangle G^0$(kJ/mol) |
|--------------|-----------------------------------------------------------------------------------|--------------------------|
| Sulfate      | CH$_4$+SO$_4^{2-}$→HCO$_3^-$+HS$^-$_+H$_2$O                                      | -16.6                    |
| Nitrate      | 5CH$_4$+8NO$_3^-$+8H$^+$→5CO$_2$+4N$_2$+14H$_2$O                                 | -756.0                   |
| Nitrite      | 3CH$_4$+8NO$_2^-$+8H$^+$→3CO$_2$+4N$_2$+10H$_2$O                                 | -928.0                   |
| Perchlorate  | CH$_4$+ClO$_4^-$_→HCO$_3^-$+Cl$^-$_+H$^+$+H$_2$O                                  | -895.9                   |
| Arsenate     | CH$_4$+4H$_2$AsO$_4^-$→CO$_2$+4H$_2$AsO$_3^-$+2H$_2$O                            | -154.0                   |
| Chromate     | 3CH$_4$+8CrO$_4^{2-}$+16H$^+$→3CO$_2$+4Cr$_2$O$_3$+14H$_2$O                      | -708.0                   |

3. Aerobic Methane Oxidation Coupled to Denitrification

In the CH$_4$-MBfR, the denitrification reaction can occur in either aerobic or anaerobic environment. Regardless of the oxygen supply regimes, biological NO$_3^-$ reduction driven by methane was ascribed to the synergistic interactions of the microbial consortium, though with varying microbial members under different dissolved oxygen concentration conditions[8]. In aerobic methane oxidation coupled to denitrification (AMO-D) process, two bacteria groups, i.e., methanotrophs (methane oxidizers) and denitrifiers, play a pivotal role in contributing to NO$_3^-$ removal[9]. Compared to conventional activated sludge reactor, the spatial distribution of methane was optimized in the biofilm of CH$_4$-MBfR, which is conductive to improving the process performance of the system. It was estimated that at a same biomass concentration, the NO$_3^-$ removal rate of CH$_4$-MBfR was approximately 4 times that of the conventional activated sludge reactor[10]. In the case of low oxygen/carbon ratio (O$_2$/CH$_4$<1), especially when the ratio was lower than 0.25, the aerobic methane-oxidizing bacteria was effectively enriched, which could facilitate the growth and metabolism of denitrifying bacteria by secretion of more organic carbon source as well as creating low oxygen environment[10].

4. Anaerobic Methane Oxidation Coupled to Denitrification

The anaerobic methane oxidation coupled to denitrification (ANMO-D) process was performed by the co-participation of an archaean group affiliated distantly to anaerobic methanotrophic archaea and a bacteria group pertaining to the candidate division NC10, in which the archaea and NC10 bacteria
dominate the reduction process of nitrate to nitrite as well as nitrite to N₂, respectively[3, 11]. Hitherto, the proven ANMO-D mechanism involves two pathways, that is, the internal oxygen-producing pathway occurring in a single microorganism belonging to NC10 bacteria (e.g., *Candidatus* Methylomirabilis oxyfera) [3] and reverse methane-producing pathway in the archaea (e.g., *Candidatus* Methanoperedens nitroreducens) [8], as delineated in Figure 2. In ANMO-D process, the doubling time of the involved microbial consortium is commonly as long as 7 to 18 months, however, it has been substantiated that by use of the hollow fiber membranes as the habitat of microorganisms can realize the shortening of the start-up period of ANMO-D process[12]. Fan[13] found that in CH₄-MBiR, the efficient enrichment of ANMO-D functional microorganisms could be achieved if the system parameters, e.g., initial nitrite concentration, operation temperature and influent pH, were adjusted to the optimal values.

**Figure 2.** The proposed two anaerobic methane oxidation pathways: A. Reverse methanogenesis, B. Intra-aerobic anaerobic oxidation[14].

### 5. Recent Advances and Research Opportunities

The removal efficiencies of CH₄-MBiR against diverse oxidized pollutants have been widely assessed in the literature as a function of a set of system operation parameters, including influent contaminant loading, gas supply pressure, dissolved oxygen concentration and hydraulic retention time etc., which contributes to the scale-up application of CH₄-MBiR in wastewater treatment practices. According to available references, by optimization of system operation parameters, the removal flux of CH₄-MBiR for NO₃⁻, TN, ClO₄⁻ and Cr²O₇⁻ could reach 684 g N/m³ per day, 1.5 kg N/m³ per day, 92.75 mg/m² per day and 33.9 mg Cr/m² per day, respectively[15-18]. Despite the involved dominant functional microorganisms[18, 19], e.g., contaminant-reducing bacteria (e.g., *Rhodocyclaceae*, *Xanthomonadaceae* and *Comamonadaceae*), fermenters (e.g., *Veillonellaceae*), aerobic methanotrophs (e.g., *Methylococcaceae* and *Methylocystaceae*), as well as the functional genes copies of particulate methane monooxygenase (*pMMO*) [9, 11, 17], have been identified, the detailed pathways in the cases of varying oxygen regimes still warrants future in-depth research by employing isotope, meta-transcriptomics and metagenomics techniques[19]. In addition, future study should lay emphasis on the improved methods of biofilm management[14], the effect of gas back-diffusion on the close-end membranes as well as the membrane durability and costs to further the development and the understanding of CH₄-MBiR for a variety of oxidized pollutants elimination[4].

### 6. Conclusion

As a promising biological technology for oxidized pollutants (particularly NO₃⁻) reduction from water,
CH4-MBfR has received widespread popularity in recent years. The operation principle of CH4-MBfR has been well-documented in the literature, and a growing body of research has been carried out with the results indicating the efficient and reliable removal efficiency of CH4-MBfR for various oxidized pollutants. Denitrifying behavior and mechanism of CH4-MBfR is one of the most researched areas, and current findings have identified the main functional microorganisms and proposed the methane oxidation pathways pertaining to the synergistic interactions of microbial consortium in AMO-D and ANMO-D processes. Nonetheless, the further development and understanding of CH4-MBfR still lie in the elucidation of in-depth mechanism and solution of cost-efficiency issues in future research.

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