Metal-dependent anaerobic methane oxidation in marine sediment: insights from marine settings and other systems

Lewen Liang[1,2], Yinzhao Wang[1,2], Orit Sivan[3] & Fengping Wang[1,2*]

[1]State Key Laboratory of Microbial Metabolism, School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, Shanghai 200240, China;
[2]Joint International Research Laboratory of Metabolic & Developmental Sciences, Shanghai Jiao Tong University, Shanghai 200240, China;
[3]Department of Geological and Environmental Sciences, Ben-Gurion University of the Negev, Beer-Sheva 84105, Israel

Anaerobic oxidation of methane (AOM) plays a crucial role in controlling global methane emission. This is a microbial process that relies on the reduction of external electron acceptors such as sulfate, nitrate/nitrite, and transient metal ions. In marine settings, the dominant electron acceptor for AOM is sulfate, while other known electron acceptors are transient metal ions such as iron and manganese oxides. Despite the AOM process coupled with sulfate reduction being relatively well characterized, researches on metal-dependent AOM process are few, and no microorganism has to date been identified as being responsible for this reaction in natural marine environments. In this review, geochemical evidences of metal-dependent AOM from sediment cores in various marine environments are summarized. Studies have showed that iron and manganese are reduced in accordance with methane oxidation in seeps or diffusive profiles below the methanogenesis zone. The potential biochemical basis and mechanisms for metal-dependent AOM processes are here presented and discussed. Future research will shed light on the microbes involved in this process and also on the molecular basis of the electron transfer between these microbes and metals in natural marine environments.

anaerobic methane oxidation, metal-AOM, marine sediment, archaea, electron transfer

Introduction

Methane is one of the most potent greenhouse gases that is mainly produced through the methanogenesis process by methanogens (Ciais et al., 2014). Anaerobic oxidation of methane (AOM) coupled with sulfate reduction is considered to be the major methane sink below the ocean floor; this process consumes up to 90% of the methane generated in anoxic marine sediments (Knittel and Boetius, 2009; Reeburgh, 2007). AOM was first observed in vertical geochemical profiles, particularly at the sulfate-methane transition zones (SMTZ) in marine sediment cores, where upward-diffusing methane is consumed coupled with sulfate depletion (Reeburgh, 1976; Iversen and Jorgensen, 1985). This was initially believed to be an abiotic process due to the extremely low energy yield available to support life (reviewed in (Knittel and Boetius, 2009)). However, through the isotope fractionation of lipid biomarker, and fluorescence *in situ* hybridization (FISH) and FISH coupled-secondary ion mass spectrometry (FISH-SIMS) analyses, it was confirmed that AOM is a microbial process, mainly conducted by the consortia consisting of anaerobic methane-oxidizing archaea (ANMEs) and sulfate-reducing bacteria (SRB) (Boetius et al., 2000; Hinrichs et al., 1999; Orphan et al.,

*Corresponding author (email: fengpingw@sjtu.edu.cn)

2001). Furthermore, in addition to sulfate, the well-known electron acceptor in SMTZ, a variety of other environmentally essential compounds such as nitrate, nitrite, and transient metal oxides (iron, manganese, and chromium) have also been found to be capable of coupling to the AOM process (Timmers et al., 2017). Sulfate and metal (iron, manganese)-dependent AOM were identified in marine settings while nitrate/nitrite and metal (iron, chromium)-dependent AOM were spotted in freshwater samples (á Norði and Thamdrup, 2014; Ettwig et al., 2010; Haroon et al., 2013; Raghoebarsing et al., 2006; Shen et al., 2016). In marine settings, whereas the microbes mediating sulfate-dependent AOM have been identified, those involved in metal-dependent AOM remain to date largely unknown.

Although identified in marine seep sediment (Beal et al., 2009), AOM process coupled with metal oxides reduction is presumed to be a challenging task for microbes in diffusive marine sediments. Under natural environmental settings in marine sediments, metal oxides exist in the form of minerals which are barely soluble and hard for microbes to use (Canfield, 1989). Based on the microbial respiration cascade, the primarily available electron acceptors such as oxygen, nitrate, manganese, iron, and sulfate are sequentially used to couple organic matter remineralization above the SMTZ in the sediment (Emerson and Hedges, 2003) (Figure 1). In this

way, relatively easily used metal oxides are reduced above the methane-rich zone, and the "survived" metal oxides are in the inert form such as the iron minerals bound in sheet silicates, which are assumed to be preserved even on time-scales of up to thousands of years (Canfield et al., 1992). However, the reduced form of metal ions such as Fe^{2+} and Mn^{2+} are usually found increasing in deep methanogenesis zones in marine sediment cores, which suggests the occurrence of reduction of metal oxides in this layer where other electron acceptors are already depleted (D'Hondt et al., 2004; Oni et al., 2015; Vigderovich et al., 2019).

Furthermore, studies on modeling have revealed that metal-dependent AOM accounts for the decrease of methane and reduction of metal oxides observed in the methanogenic zone. A metal-dependent AOM zone in deep marine sediment (Figure 1) (Riedinger et al., 2014; Sivan et al., 2007) as well as in brackish coastal sediments (Egger et al., 2017; Egger et al., 2016; Egger et al., 2015; Rooze et al., 2016; Segarra et al., 2013; Slomp et al., 2013), has been proposed. However, the responsible microbes and the strategies they employ for metal-dependent AOM in the marine environment are still unknown. In this mini-review, we summarize the geochemical evidences from sediment cores in various marine environments and discuss potential biochemical processes involved in metal-dependent AOM. In addition,
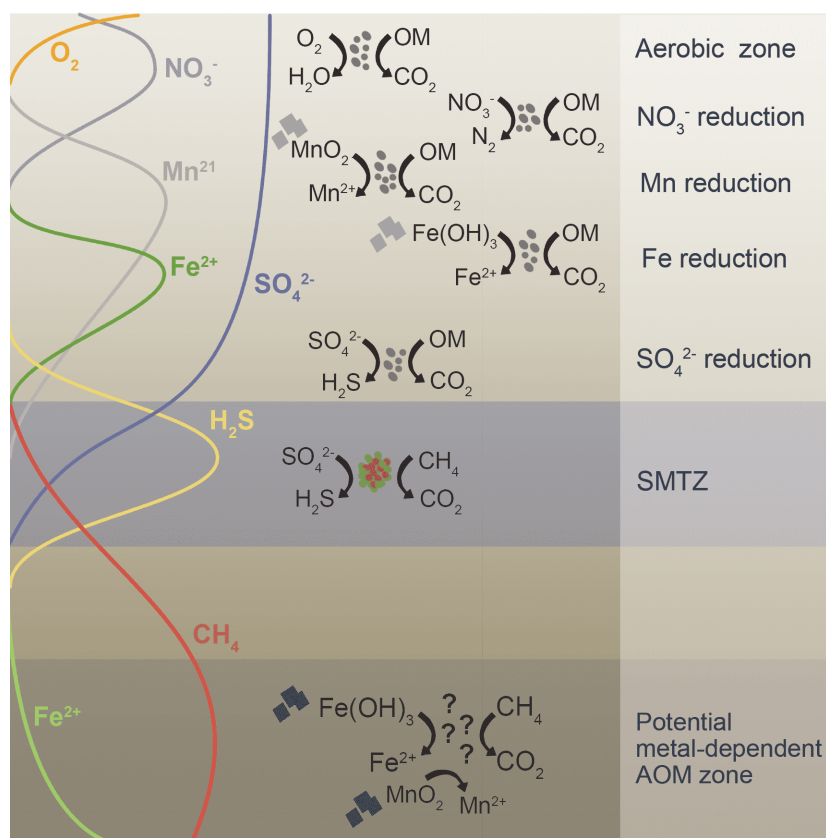

Figure 1 (Color online) Sequential utilization of electron acceptors in marine sediment (modified from Emerson and Hedges, 2003; Riedinger et al., 2014).

we discuss the microbes which are possibly responsible for these processes and their metabolic properties and call for future research attention in this regard.

Geochemical evidence of metal-dependent AOM in marine sediment

The first documented evidence of potentially metal-driven AOM could be dated back to the year 1980. The presence of iron and manganese in microcosm incubation systems was found to increase the ratio of methane oxidation to methane formation as revealed by isotopic data (Zehnder and Brock, 1980), suggesting that these metal ions may stimulate the AOM process. Although sulfate was the only known electron acceptor for AOM in marine sediment at the time, accumulating geochemical profiles revealed the decoupling of methane oxidation from sulfate reduction in anoxic marine sediment; suggesting the presence of other electron acceptors such as iron and manganese (Hoehler et al., 1994). Besides, high concentrations of reduced metal ions were also observed below the SMTZ in the deep methanogenic zone, which implies the existence of an unrecognized relationship between metal reduction and methane cycling (D'Hondt et al., 2004). Diffusion and reaction simulation of the pore water profiles from West African Margin deep sea sediment further indicates metal-dependent AOM as the possible process for methane decrease in the methanogenic zone (Sivan et al., 2007).

In the year 2009, Beal et al. in their study conducted a microcosm experiment by adding metallic minerals (birnessite and ferrihydrite) to the marine seep sediment collected from the Eel River Basin and revealed the potential for metal (manganese and iron)-dependent AOM in a marine environment (Beal et al., 2009). Sivan et al. (2014) in their study showed that iron-dependent AOM in these seeps could occur concurrent with sulfate-dependent AOM (Sivan et al., 2014). Additionally, a study of the decoupling of AOM and sulfate reduction in the incubated seep sediments from the Santa Monica Basin revealed that ferric citrate could be used as an alternative electron acceptor (Scheller et al., 2016).

The AOM process usually causes higher alkalinity and increasing concentration of dissolved inorganic carbon in pore waters and leads to precipitation of authigenic carbonates as well as the formation of carbonate deposits (Chen et al., 2014). By combining nanoscale secondary ion mass spectrometry ion mapping and transmission mode dual energy analyses, Peng et al. in their study found that iron is widely distributed in the veins of the carbonate pipe in the northern Okinawa Trough (OT), which indicates the co-precipitation of iron with carbonate during methane oxidation. Because the organic matter content in this *in situ* environment was poor (~0.5 wt%), iron-dependent AOM has recently been proposed as one of the driving forces in the formation of the iron-rich carbonate pipe (Peng et al., 2017). In addition, similar iron or other metals-rich carbonates have also been found in the northern OT, Black Sea, South China Sea, and Gulf of Cadiz cold-seep environments (Han et al., 2013; Merinero et al., 2012; Reitner et al., 2005b; Sun et al., 2015; Tong et al., 2013); which suggests extensive distribution of metal-dependent AOM in natural marine sediments.

However, metal-dependent AOM in the deep methanogenic zone of diffusive marine sediments has mainly been considered by modeling approaches. Additional to the early modeling studies already mentioned (Sivan et al., 2007), geochemical profiles of the Argentine Basin sediment showed that microbial iron reduction occurs below the SMTZ, and it has also been suggested that the low reactivity of organic matters precludes the possibility of organo-clastic dissimilatory iron reduction and thus iron-dependent AOM in the methanogenic zone is likely to happen (Riedinger et al., 2014). Meanwhile, measured manganese usually exhibits similar behavior as iron; inferring that manganese-dependent AOM is also likely to occur at the same layer (Riedinger et al., 2014; Treude et al., 2014). A one-dimensional reactive transport model was developed for the Baltic Sea sediment sample to investigate the contribution of iron-dependent AOM as a source of ferrous iron, which is abundant in pore water (Egger et al., 2017). The model includes physical transport as well as biogeochemical transformations, assuming that iron-dependent AOM is the dominant iron oxide reduction process and that fits well with both the pore water and solid phase profiles.

Biogeochemical consideration of metal-dependent AOM

Thermodynamically, the sustainability of a living cell requires energy to synthesize adenosine triphosphate (ATP) for metabolism. Moreover, it has been considered that a favorable metabolic reaction must provide at least 15 kJ mol^{-1} energy per ATP generation (Caldwell et al., 2008). Due to its low energy yield (16.6 kJ mol^{-1}), sulfate-dependent AOM has been considered for a long time to be a thermodynamically unfavorable process in natural environments. However, it has been proposed that cells that adapt to energy-deficient conditions may require even less energy than expected to sustain life (Knab et al., 2008). Table 1 shows a list of the potential Gibbs free energy changes of the AOM process coupled to different electron acceptors. However, the identified electron acceptors of the AOM process in marine settings are restricted to sulfate, iron, and manganese. Compared to the prevalent sulfate-dependent AOM ($\Delta G^{0'}$ = -16.6 kJ mol^{-1}), which serves as an example of how life

operates close to the thermodynamic limit, the metal-dependent AOM is thermodynamically favorable (iron-dependent AOM $\Delta G^{0'}=-81.6$ kJ mol^{-1}; manganese-dependent AOM $\Delta G^{0'}=-494.0$ kJ mol^{-1}) (He et al., 2018).

However, in the *in situ* environment, the Gibbs free energy (*in situ* ΔG in Table 1) is quite different from the $\Delta G^{0'}$ calculated under standard conditions for biological processes. Furthermore, the total energy yield should include the reaction rate in the *in situ* environment. In the microcosm experiment for metal-dependent AOM using the Eel River Basin seep sediment, it was found that in the *in situ* condition, the birnessite-dependent AOM has the potential to gain 10 times more energy, while ferrihydrite-dependent AOM gains twice as much energy compared to that of sulfate-dependent AOM (Beal et al., 2009).

Additional to the direct metal-dependent AOM, metal oxides can indirectly influence the AOM process coupled with other electron acceptors. Moreover, it has been shown that the addition of hematite to the Hydrate Ridge seep sediment results in iron reduction co-occurring with increasing sulfate-dependent AOM rates (Sivan et al., 2014). Besides, the redox reaction between sulfur and iron is assumed to be similar to that in the "cryptic sulfur cycle" (Holmkvist et al., 2011; Pellerin et al., 2018). It was speculated that either the iron oxides reduction coupled with sulfur disproportionation could stimulate sulfate recycling, thus increasing the rates of sulfate-dependent AOM, or that the precipitation of pyrite could remove the end product sulfide in the system, and consequently accelerates the process.

Microbial processes in metal-dependent AOM

Known microbial processes in AOM

At present, the majority of the known microbes responsible for AOM are ANMEs in the Archaea domain, this being with the exception of NC10 bacteria which are capable of nitrite-dependent AOM (Ettwig et al., 2010) and other methanogens, as well as aerobic bacteria, that are suggested conducting iron-dependent AOM in lake sediments (Bar-Or et al., 2017). Based on 16S ribosomal RNA (rRNA) gene phylogeny, all ANMEs belong to the archaeal phylum Euryarchaeota in the Archaea domain and cluster closely with the cultivated methanogens in the class Methanomicrobia (Knittel and Boetius, 2009). ANMEs that are responsible for sulfate-dependent AOM can be divided into three groups: ANME-1 (subgroups a and b); ANME-2 (subgroups a, b, and c); and ANME-3, with 16S rRNA gene similarity between 75%–92% (Knittel and Boetius, 2009). Nitrate-dependent AOM was carried out by ANME-2d lineage enriched from freshwater systems (Haroon et al., 2013; Hu et al., 2009; Raghoebarsing et al., 2006), and was later found to be capable of catalyzing iron-dependent AOM (Ettwig et al., 2016). Recently, a new microorganism in ANME-2d lineage, which is also enriched from freshwater sediment, was found capable of iron-dependent AOM (Cai et al., 2018).

In marine sediments, ecological niche separation of ANMEs usually occurs where ANME-2a/b dominates upper layers and ANME-2c and/or ANME-1 take over in deeper zones, which indicates the diversity/specificity of metabolic capabilities of ANMEs (Niu et al., 2017). ANMEs that are responsible for sulfate-dependent AOM tend to live in syntrophy with bacterial partners and form cell consortia covered by a thick siliceous envelope (Chen et al., 2014). However, the syntrophic pattern also varies among the ANMEs. For example, ANME-1 and ANME-2 archaea are usually associated with sulfate-reducing bacteria (SRB) of the *Desulfosarcina/Desulfococcus* (DSS) clade within the Deltaproteobacteria, while ANME-3 also formed consortia with the SRB of branch *Desulfobulbus* in addition to the syntrophy with DSS (Knittel and Boetius, 2009). Further, ANME-2 was also identified to form aggregates with non-SRB partners such as *Limnobacter* spp. of Betaproteobacteria (Chen et al., 2016; Pernthaler et al., 2008). Besides, ANME-1 in the Black Sea microbial mat was found to be living without a bacterial partner (Reitner et al., 2005a).

ANMEs perform AOM through the reverse methanogenesis pathway. ANMEs and methanogens share the same sets

Table 1 Gibbs free energy changes under standard conditions ($\Delta G^{0'}$) and the calculated *in situ* ΔG of different electron acceptors (based on He et al., 2018; Lu et al., 2016; Beal et al., 2009)

Reaction	$\Delta G^{0'}$ (kJ mol^{-1} CH$_4$)	*In situ* ΔG (kJ mol^{-1} CH$_4$)
$CH_4 + SO_4^{2-} \rightarrow HCO_3^- + HS^- + H_2O$	-16.6	$-14\sim-35$
$CH_4 + 8Fe(OH)_3 + 15H^+ \rightarrow HCO_3^- + 8Fe^{2+} + 21H_2O$	-81.6	-270.3
$CH_4 + 4MnO_2 + 7H^+ \rightarrow HCO_3^- + 4Mn^{2+} + 5H_2O$	-494.0	-556
$CH_4 + 4NO_3^- \rightarrow CO_2 + 4NO_2^- + 2H_2O$	-519.8	N/A
$CH_4 + 4/3Cr_2O_7^{2-} + 32/3H^+ \rightarrow 8/3Cr^{3+} + CO_2 + 22/3H_2O$	-878.8	N/A
$CH_4 + 8/3NO_2^- + 8/3H^+ \rightarrow CO_2 + 8/3N_2 + 10/3H_2O$	-928	N/A

of enzymes for methane metabolism while working in different directions (Hallam et al., 2004). This assertion has been verified by the ANME-2a genome that contains all the required genes that satisfy this hypothesis (Wang et al., 2014). In this pathway, methane is activated by the methyl-coenzyme M reductase (MCR), and then finally oxidized to CO_2 (McGlynn, 2017). MCR is considered to be the key enzyme in methane and short-chain alkanes (such as propane and n-butane) metabolism for archaea (Scheller et al., 2010; Laso-Pérez et al., 2016). However, genes that encode the MCR complex are also found to exist in genomes of the phyla Bathyarchaeota (formerly known as the Miscellaneous Crenarchaeota Group (MCG) (Meng et al., 2014)), Verstraetearchaeota, and other archaeal phyla. Although the functions of the MCR complex encoded in these archaeal genomes have not been confirmed, it has opened the possibilities of methane/short-chain alkane metabolism outside the Euryarchaeota phylum, and indicating versatile methane/ short-chain alkane metabolic properties (Berghuis et al., 2019; Borrel et al., 2019; Boyd et al., 2019; Colman et al., 2019; Evans et al., 2015; McKay et al., 2019; Seitz et al., 2019; Vanwonterghem et al., 2016; Wang et al., 2019a; Wang et al., 2019b).

Microorganisms participate in metal-dependent AOM

To the best of our knowledge, evidence of microbes that are potentially responsible for metal-dependent AOM in marine samples is very little. One of these evidences is based on the seep sediment collected from the Eel River Basin; where after 10 months enrichment using manganese (birnessite) as the electron acceptor, a shift of the microbial community was observed compared to the original sample and control groups. Based on the community shift during the enrichment, the authors of this study suggested that either ANME-1 or methanococcoides/ANME-3 with a bacterial partner might be responsible for the metal-dependent AOM (Beal et al., 2009). Furthermore, another study on the methane seep sediment of the Santa Monica Basin revealed that the enriched samples which contained high abundance of ANME-2a and ANME-2c and relatively low abundance of ANME-1 could decouple the AOM process from SRB activities when ferric iron compounds (ferric citrate and ferric-EDTA) were added (Scheller et al., 2016). Research on the North Sea sediment showed that members of the candidate division JS1, methanogens and Methanohalobium/ANME-3-related archaea, are closely linked to the profile of the dissolved iron in the methanogenic sediments, as revealed by the result of the correlation analysis between the microbial populations and geochemical profiles in sediment cores. This indicates their potential involvement in iron-dependent AOM in marine environments (Oni et al., 2015).

In freshwater systems, ANME-2d has been found to be capable of performing metal-dependent AOM. An enrichment culture of denitrifying anaerobic methane oxidation, containing ANME-2d and the bacterial partner, are shown to separately support iron and manganese-dependent AOM (Ettwig et al., 2016) and chromium-dependent AOM (Lu et al., 2016). Also, long-term enrichment fed with ferrihydrite and methane revealed that most of the archaea belong to ANME-2d; which further indicates iron-dependent AOM (Cai et al., 2018). Besides, a much more complex interplay between methanogens and methanotrophic bacteria has also been suggested to be responsible for the iron-dependent AOM in lake sediment (Bar-Or et al., 2017). The authors in this research set up a long-term incubation, adding $^{13}CH_4$ and iron minerals to the systems; where the incorporation of ^{13}C into the fatty acids and the *pmoA* gene increase indicated the involvement of methanotrophic bacteria (probably not NC-10), while inhibition of activity of methanogens (probably not ANMEs) completely stopped iron-dependent AOM in the system.

Potential metabolic pathways for metal-dependent AOM

Since there is no representative of metal-dependent AOM microbes from the marine environment, it is still unclear which pathway is involved. Nevertheless, genome reconstruction of ANME-2d from freshwater sediment enrichment that is capable of iron-dependent AOM possesses all the genes for the "reverse methanogenesis" pathway, which are highly expressed, as revealed by metatranscriptomic data (Figure 2A) (Cai et al., 2018). Besides, a recent biochemical study has revealed that *Methanosarcina acetivorans*, a cultured methanogen, could reduce iron during the oxidation of methane using the reverse methanogenesis pathway as ANME-2a (Figure 2B) (Yan et al., 2018). Nevertheless, how the electron is transferred to ferric iron remains to date unknown. In most ANMEs, multi-heme c-type cytochromes (MHCs) serve as a possible electron shuttling. This has already been observed in iron-reducer *Geobacter sulfurreducens* that use MHCs to transfer electrons during Fe (III) and Mn (IV) oxide reduction (Mehta et al., 2005), and might also work for metal-dependent AOM. MHCs have been inferred to be involved in direct interspecies electron transfer for exchange reducing equivalents between the syntrophic AOM consortium (McGlynn et al., 2015; Wegener et al., 2015). High numbers of MHCs are present and are highly expressed in ANME-2d from freshwater iron-dependent AOM enrichment, including those located adjacent to the menaquinone gene and the one that contains an S-layer domain, indicating its capability to perform electron transfer from cytoplasmic membrane to iron oxides (Cai et al., 2018). Biochemical data of *Methano-*
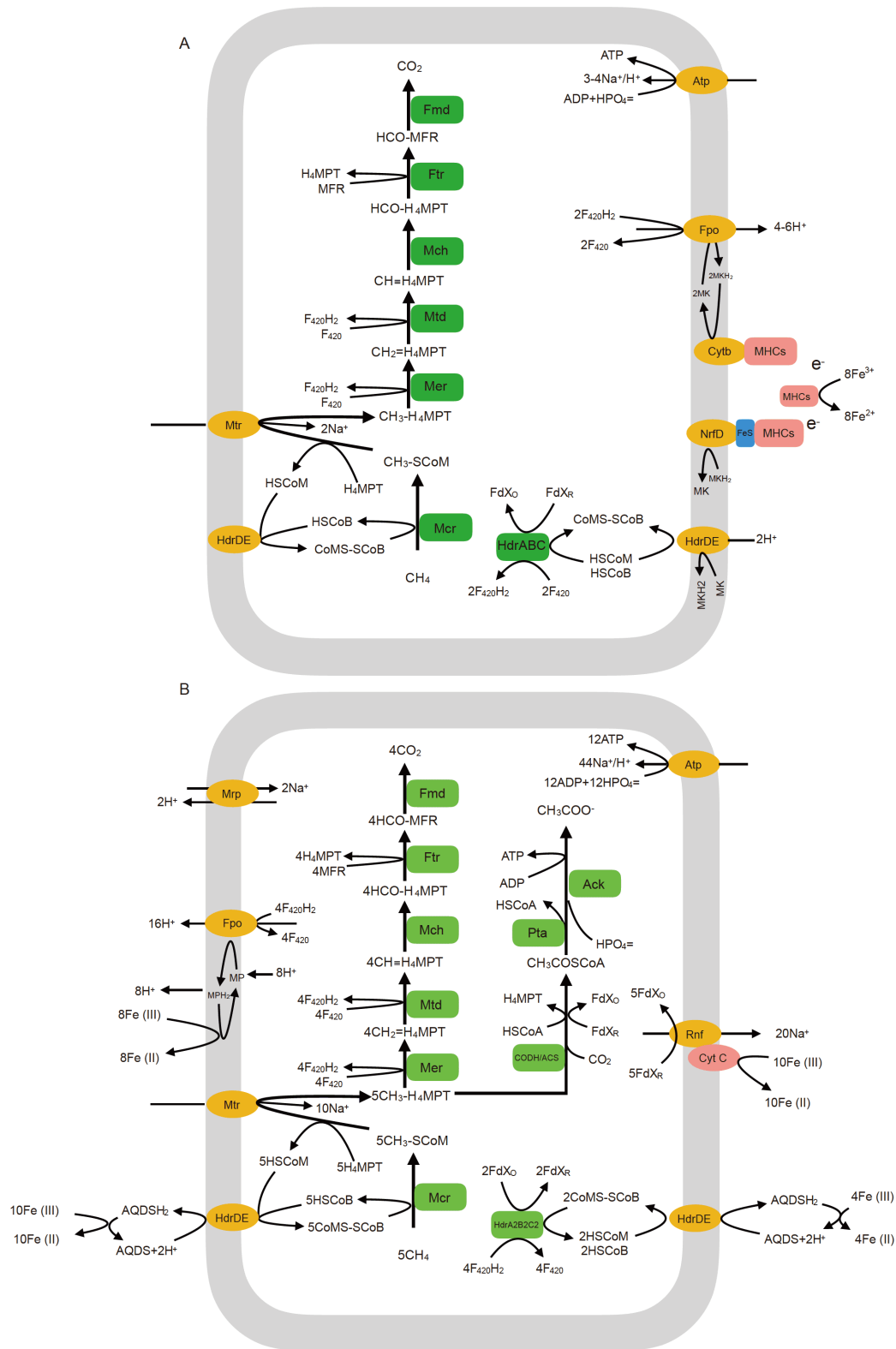
Figure 2 (Color online) A proposed metabolic pathway of iron-dependent AOM inferred from the genomes of ANME-2d. A, ANME-2a and *Methanosarcina acetivorans*; (B, Abbreviations for major enzymes and co-factors. Mcr, Methyl-coenzyme M reductase; Mtr, Tetrahydromethanopterin S-methyltransferase; Mer, Coenzyme F_{420}-dependent methylene-H_4MPT reductase; Mtd, F_{420}-dependent methylene-H_4MPT dehydrogenase; Mch, methenyl-H_4MPT cyclohydrolase; Ftr, formylmethanofuran: H_4MPT formyltransferase; Fmd, Formylmethanofuran dehydrogenase; CODH/ACS, CO dehydrogenase/acetyl-CoA synthase; Pta, phosphotransacetylase; Ack, acetate kinase; Fd, ferredoxin; Hdr, heterodisulfide reductase; Cytb, b-type cytochrome; NrfD, polysulfide reductase subunit D; FeS, ferredoxin iron-sulfur protein; Fpo, $F_{420}H_2$ dehydrogenase; Mrp, multi-subunit type Na^+/H^+ antiporter; Atp, ATP synthase. MK, menaquinone; MP, methanophenazine. Readers may refer to the paper text and references for details (Cai et al., 2018; Wang et al., 2014; Yan et al., 2018).

sarcina acetivorans revealed that methanogen enables iron-dependent AOM, and also showed that MHCs along with the low-molecular-mass humic acids- tested by analog anthra-quinone-2, 6-disulfonate (AQDS)- are the potential direct electron donor to iron oxides as shown in Figure 2B.

Outlook

Large amounts of transient metals are provided to the ocean annually (Poulton and Raiswell, 2000); for example, the global input of iron to the ocean is 703.5~1217.5 Tg per year (Jickells et al., 2005). Since transient metal elements may go through recycling hundreds of times before they are finally buried in the seabed, metal-dependent AOM potentially has a great impact on methane removal in marine sediment (Beal et al., 2009). Besides, during the Archean Eon of earth's history, anaerobic metabolic processes related to iron cycling were thought to be one of the driving forces shaping the biosphere (Canfield et al., 2006). As of then, supporting evidence has insinuated the involvement of metal-dependent AOM as the main methane sink (Beal et al., 2009; Crowe et al., 2011; Riedinger et al., 2014). However, metal-dependent AOM in marine settings has not been studied extensively. Despite accumulating geochemical evidence and modeling studies indicating the presence of metal-dependent AOM in marine seep environments, the geological prevalence and distribution of this process in the ocean is yet to be determined. Besides, as shown in Figure 1, metal-dependent AOM is likely to occur in the deep methanogenic zone, which is usually overlooked on either methane oxidation or metal reduction. The biochemical mechanism on metal-dependent AOM as well as its contribution to global carbon and metal elements cycling has not yet been studied. Due to the ecological niche separation for different clades of ANMEs as suggested earlier, microbes for metal-dependent AOM in the methanogenic zone may have distinct metabolic properties either to survive or carry out this process. As in the early age of the study of sulfate-dependent AOM, microbial studies such as enrichment and metabolic analysis are crucial in order to fully understand the underlying process. The importance and contribution of metal-dependent AOM to methane sink as well as deep-biosphere element cycling needs further investigation.

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