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Minireview

Anaerobic microbial methanol conversion in marine sediments

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Summary

Methanol is an ubiquitous compound that plays a role in microbial processes as a carbon and energy source, intermediate in metabolic processes or as end product in fermentation. In anoxic environments, methanol can act as the sole carbon and energy source for several guilds of microorganisms: sulfate-reducing microorganisms, nitrate-reducing microorganisms, acetogens and methanogens. In marine sediments, these guilds compete for methanol as their common substrate, employing different biochemical pathways. In this review, we will give an overview of current knowledge of the various ways in which methanol reaches marine sediments, the ecology of microorganisms capable of utilizing methanol and their metabolism. Furthermore, through a metagenomic analysis, we shed light on the unknown diversity of methanol utilizers in marine sediments which is yet to be explored.

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Introduction

Marine sediments are rich in biomass and a source of unknown microbial diversity, with microbial cell densities as high as 10^9 cells per cubic centimetre (up to five orders of magnitude higher than the water column) (Jørgensen and Boetius, 2007). Marine sediments consist of deposits of clay, decaying organic matter, calciferous remains and other solids. While oxygen can diffuse in these sediments, it is rapidly consumed by aerobic organisms, in an oxic layer ranging from a few millimetres to several meters in depth, depending on a variety of factors such as organic matter input to sediment surface, sediment permeability, turbation by water currents or macrofauna, water column height, microbial activity or proximity to continental shelves (Glud, 2008; D'hondt *et al*., 2015). The underlying sediment remains anoxic, where microbial fermentation and anaerobic respiration are the main metabolic processes. Molecules containing no carbon–carbon bonds such as trimethylamine, dimethylsulfide, methane and methanol are suggested to be important energy sources for microorganisms in these environments (Yanagawa *et al*., 2016; Chistoserdova and Kalyuzhnaya, 2018; Sun *et al*., 2019). As the fermentation products of common osmolytes or carbohydrates, these compounds are widely present in marine systems. Besides converted by microbial activity, they can influence the climate as atmospheric aerosols and as such, their role in marine environments is in general well-reviewed (Reisch *et al*., 2011; Lidbury *et al*., 2017; Timmers *et al*., 2017; Sun *et al*., 2019). However, there is a lack of information on the microbial utilization of methanol in anoxic marine sediments. In this review, we aim to present what is currently known about the presence and fate of methanol in anoxic marine sediments. To provide insight into anaerobic microbial methanol utilization in diverse marine sediments, we performed a metagenome mining of 246 published metagenomes of anoxic marine sediments for key methanol utilization genes. This effort reveals the ubiquitousness of several genes involved in anaerobic methanol conversion in marine sediments,

further supporting the importance of methanol-utilizing microorganisms in these environments.

Sources of methanol in marine sediments

Several studies have quantified methanol concentrations in marine systems, both in water columns and sediments. Concentrations in the water column range from less than 27 nM up to 429 nM in marine systems, although estimates vary widely even between studies on the same site (Williams *et al*., 2004; Kameyama *et al*., 2010; Dixon *et al*., 2011a,b; Dixon and Nightingale, 2012; Read *et al*., 2012; Yang *et al*., 2013). It should be noted, however, that most of these studies focused solely on the Atlantic Ocean and mainly on shallow, aerobic subsurface waters, with only one study investigating a gradient of up to 500 m depth (Dixon and Nightingale, 2012). Estimates of methanol levels in coastal area sediments range between 0.3 μM and over 100 μM in environments as diverse as the Orca Basin in the Gulf Mexico, East Japan Sea and the South China Sea (Yanagawa *et al*., 2016; Zhuang *et al*., 2014; 2019a,b). In these studies, methanol concentrations were found to increase with depth, with the lowest concentrations close to the seafloor and the highest concentrations at depths of 10–20 m below the seafloor. This increase in methanol with depth is attributed to higher methanol turnover to CO_2 near the sediment-water column interphase (Zhuang *et al*., 2019a,b). Table 1 gives an overview of marine anaerobic sediments where methanol concentrations have been measured.

Methanol sources in marine systems are attributed to both *in situ* production and external depositions from terrestrial origins. Terrestrial methanol mainly originates as a by-product of plant growth and to a lesser degree through fermentation of pectin (Jacob *et al*., 2005; Millet *et al*., 2008). Because of its volatility, terrestrially produced methanol evaporates into the atmosphere, with an estimated annual emission of 70–350 Tg (Galbally and Kirstine, 2002). A large amount of this methanol, estimated between 8 and 101 Tg $year^{-1}$ is deposited in the oceans through air–sea exchange, diffusion and rainfall (Beale *et al*., 2013). It should be noted that higher estimations of methanol deposition in the oceans also take sea to air emissions into account, which is estimated to be 30–85 Tg $year^{-1}$, thereby diminishing total methanol deposition in the oceans (Heikes *et al*., 2002; Dixon *et al*., 2011a,b; Dixon and Nightingale, 2012; Beale *et al*., 2013; Yang *et al*., 2013).

Methanol is also produced *in situ* in the oceans. A study of methanol production in Atlantic waters estimated a net production of around 49 nmol L^{-1} day^{-1} (Dixon *et al*., 2013). Sources of this methanol are primary production by phytoplankton where methanol is an exudate by-product and through microbial fermentation of algal carbohydrates such as galactins and pectin (Sieburth and Keller, 1989; Riemer, 1998; Dixon *et al*., 2013). Phytoplankton accounts for almost half of the global primary production and as such is suspected to be a major contributor to marine methanol production, the same equivalent to terrestrial primary production (Cloern *et al*., 2014; Mincer and Aicher, 2016). Interestingly, there have been very few studies quantifying net methanol production by phytoplankton. Mincer and Aicher (2016) assessed methanol production through ^{13}C-labelled bicarbonate addition to axenic phytoplankton cultures. As much as 0.3% of all assimilated carbon was sequestered in methanol, and it was implied that the genus *Prochlorococcus* alone could produce 846–1693 Tg of methanol per year, worldwide (Neufeld *et al*., 2008; Mincer and Aicher, 2016). Furthermore, phytoplankton mobilizes between 10% and 35% of their assimilated carbon into pectin, lignin and galactans, which are methoxylated polysaccharides (Sista Kameshwar and Qin, 2018). Thus, through

Table 1. Measured levels of marine sediment methanol concentrations.

Location	Depth	Methanol concentration	Reference
Black Sea sediment	0–700 cm below seafloor	6 μM at sea floor 1 μM 100–400 cm bsf 6 μM 500 cm bsf	Zhuang *et al*. (2014)
Northern Gulf of Mexico	0–30 cm below seafloor	2 μM at sea floor 65 μM 30 cm bsf	Zhuang *et al*. (2014)
South China Sea	0–700 cm below seafloor	4.3 μM at sea floor 111.7 μM at 700 cm bsf	Zhuang *et al*. (2019a,b)
Umitaka Spur, eastern Japan Sea	0–35 m below seafloor	0.3–3.5 μM at 0–3 m bsf 20 μM at 30 m bsf	Yanagawa *et al*. (2016)
Intertidal sediment, Lowes Cove, Maine	Seafloor	0.5–3.5 μM	King *et al*. (1983)
Guaymas Basin, Gulf of California	0–40 cm below seafloor	0.2–2 μM at seafloor 36.7 μM at 35 cm bsf	Zhuang *et al*. (2019a,b)
Western Mediterranean Sea	0–500 cm below seafloor	0.5–1.5 μM across all depths	Zhuang *et al*. (2018a,b)

demethoxylation of these carbohydrates by both aerobic microorganisms in the water column and oxic sediment and anaerobic microorganisms in the anoxic sediment, methanol is released (Schink and Zeikus, 1980, 1982; Sieburth and Keller, 1989; Sista Kameshwar and Qin, 2018).

Chemolithotrophic microorganisms present in anaerobic sediments are capable of producing methanol. Anaerobic oxidation of methane occurs in most anoxic systems where methane is present, by methane-oxidizing microorganisms or syntrophic microbial communities capable of using sulfate, nitrate, manganese or iron as terminal an electron acceptor (Wegener *et al*., 2016; Welte *et al*., 2016; McGlynn, 2017; Timmers *et al*., 2017; Leu *et al*., 2020). Canonically, anaerobic methane oxidizers can metabolize methane through a reversal of the methanogenesis process (Haroon *et al*., 2013). This process, contrary to oxic methanotrophy (which relies on the activity of oxygen-dependent methane monooxygenase), does not involve methanol as intermediate (Cicerone and Oremland, 1988; Oremland and Culbertson, 1992). However, in freshwater sediments, methane oxidation by nitrate- and nitrite-dependent facultative anaerobic organisms has been shown to occur via particulate methane monooxygenase, with methanol as a key intermediate (Grinsven *et al*., 2020). *Ca*. Methylomirabilis oxyfera utilizes a strategy of intracellular production of oxygen from nitrite. This oxygen can then act as electron acceptor for methane oxidation, also utilizing particulate methane monooxygenase (Wu *et al*., 2011; Timmers *et al*., 2017; Vaksmaa *et al*., 2017). These processes with methanol as intermediary are leaky, and diffused methanol can be used by surrounding methylotrophic microorganisms (Wu *et al*., 2011; Chistoserdova and Kalyuzhnaya, 2018). Thus far, these processes have only been described in freshwater environments, which are richer in nitrite and nitrate than marine sediments (Ettwig *et al*., 2009; Grinsven *et al*., 2020). However, in an enrichment culture of marine origin with methane as substrate, the NC10 phylum to which *Ca*. M. oxyfera belongs was abundant (He *et al*., 2015). Furthermore, phylogenetic studies on marine oxygen minimum zones report detection of 16S rRNA genes closely related to NC10 bacteria as well as transcripts of particulate methane monooxygenase and nitric oxide reductase genes in oxygen minimum zones off the coast of northern Mexico and Costa Rica (Padilla *et al*., 2016). These findings suggest an environmental role of the NC10 phylum in marine environments as well.

It is unclear if and how methanol produced in surface water or exchanged with the air reaches the sediment of marine systems. As discussed above, methanol levels in the sediment are substantially higher than in the water column. Gradual deposition of methanol in the water column into the sediment is possible, but relatively high turnover rates of methanol in the water column (<1 day) suggests metabolization before it reaches anoxic sediments (Dixon *et al*., 2011a,b). This high methanol turnover can also explain its lower concentrations in the water column (Dixon *et al*., 2011a,b). In marine sediments, methanol turnover is estimated to be between 22 and more than 100 days (Zhuang *et al*., 2019a,b).

Biochemistry of microbial methanol utilization

In anaerobic environments, methanol can be converted by three distinct processes: oxidation to CO_2 (e.g. by sulfate-reducing microorganisms (SRM), Equation 1), conversion with CO_2 to acetate (by acetogens, Equation 2), or conversion to methane and CO_2 (by methanogens, Equation 3) (Goorissen *et al*., 2004; Chistoserdova, 2015; Yanagawa *et al*., 2016, Gibbs free energy changes calculated with data from Thauer *et al*., 1977). The distinct pathways employed for methanol conversion by these organisms are described below and are summarized in Fig. 1.

$$CH_3OH + 3\,SO_4^{2-} \rightarrow 4\,HCO_3^- + 3\,HS^- + 4\,H_2O + H^+ \quad -364\,kJ/reaction \tag{1}$$

$$4\,CH_3OH + 2\,HCO_3^- \rightarrow 3\,C_2H_3O_2^- + 4\,H_2O + H^+ \quad -221\,kJ/reaction \tag{2}$$

$$4\,CH_3OH \rightarrow 3\,CH_4 + CO_2 + 2\,H_2O \quad -311\,kJ/reaction \tag{3}$$

The methanol methyltransferase system is a major pathway for methanol metabolism in anoxic environments. This pathway is catalysed by the methanol:coenzyme M methyltransferase MtaABC, found in methanogens, acetogens and SRM (Sauer *et al*., 1997; Visser *et al*., 2016; Sousa *et al*., 2018). Subunit MtaB cleaves the C-O bond of the methanol and transfers the methyl group to a second subunit, MtaC, using cobalamin as a cofactor (Sauer *et al*., 1997). MtaC requires the cobalamin to contain the highly reduced cobalt(I), which is only possible in strict anoxic conditions (van der Meijden *et al*., 1984; Daas *et al*., 1996). The methylated group is subsequently transferred to either coenzyme-M (HS-CoM) in methanogens or tetrahydrofolic acid (THF) in acetogens and SRM by MtaA, forming CH_3-CoM or CH_3-THF respectively (van der Meijden *et al*., 1983; Stupperich, 1994; Daas *et al*., 1996; Sousa *et al*., 2018; Evans *et al*., 2019). It has been proposed that MtaA can be replaced with THF-methyltransferase in *Sporomusa* strain An4 (Visser *et al*., 2016).

In methanogenic methanol conversion, one-quarter of the substrate is oxidized to CO_2 through a reversal of the hydrogen/CO_2 methanogenesis pathway. This process

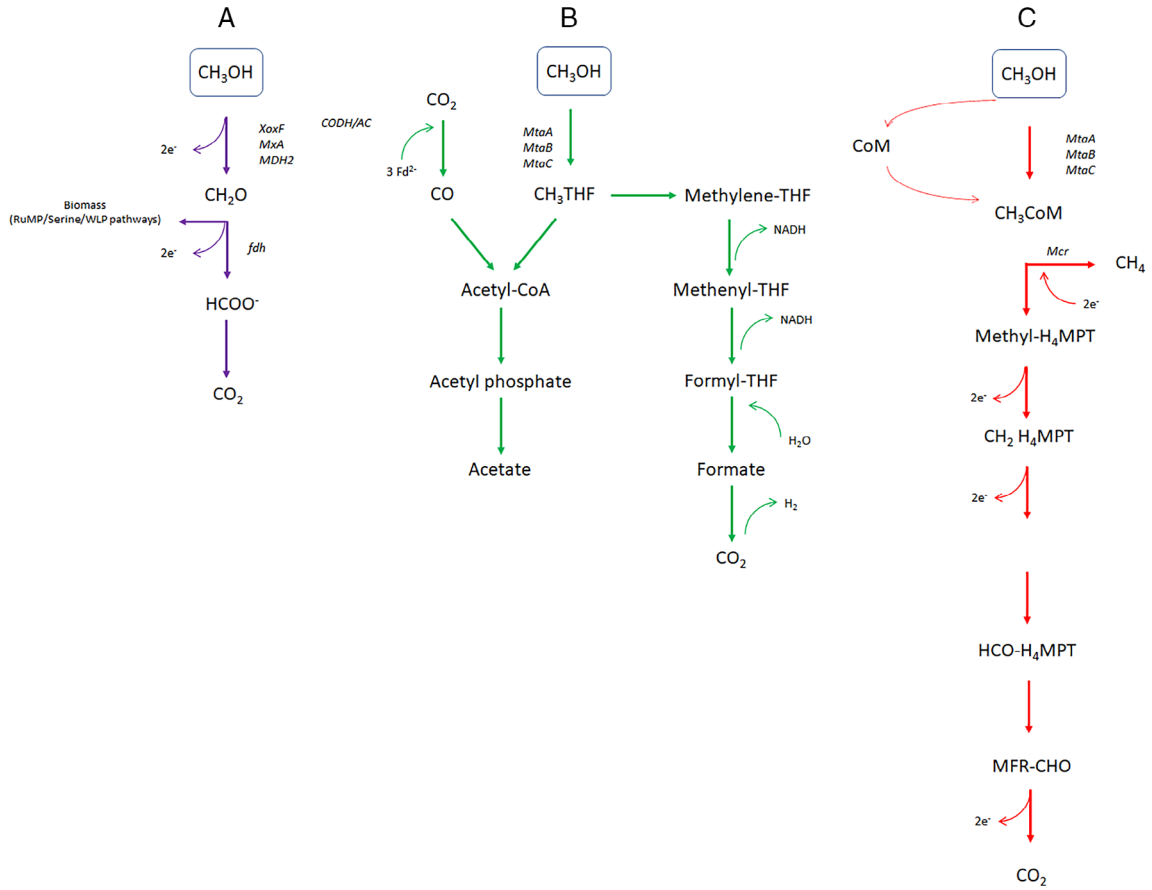

Fig. 1. Methanol degradation pathways as outlined in this review.
A. Respiratory methanol oxidation. Abbreviations: *xoxF*, lanthanide-dependent methanol dehyrdogenase; *MxA*, calcium-dependent methanol dehydrogenase; *RuMP pathway*, ribulose monophosphate pathway for carbon fixation; *WLP*, Wood–Ljungdahl pathway for carbon fixation.
B. Acetogenesis pathway. Abbreviations: *THF*, tetrahydrofolic acid; *CODH/ACs*, carbon monoxide dehydrogenase/Acetyl-coA synthetase
C. methanogenesis; *MT*, methyl-transferase 1; *CoM*, co-enzyme M; *H_4MPT*, tetrahydromethanopterin; *Mcr*, methyl-coenzyme M reductase; *MFR*, methanofuran. [Color figure can be viewed at wileyonlinelibrary.com]

yields six electrons per molecule of methanol, generating sufficient reducing power to reduce the remaining three-quarters of the substrate, which is shuttled to CoM-CH_3. This CoM-CH_3 is further reduced by methyl coenzyme M reductase (Mcr) to methane and HS-CoM (Gottschalk and Thauer, 2001; Welander and Metcalf, 2005). Furthermore, some methanogens of the order *Methanomassiliicoccales* couple H_2 oxidation to methanol reduction, yielding solely methane as product (Costa and Leigh, 2014; Lang *et al*., 2015; Nobu *et al*., 2016).

In acetogens and SRM generated CH_3-THF can be integrated into the Wood–Ljungdahl pathway (WLP, Fig. 1). One in four molecules of CH_3-THF is used in a reversal of the methyl branch of the WLP, oxidizing the methylated group to CO_2 and generating 2 mol of NAD(P)H, 1 mol of ATP and 1 mol of H_2. The generated H_2 is then utilized in a bifurcating mechanism to generate 0.5 mol of reduced ferredoxin (Fd^{2-}). The microorganisms invest ATP to produce a proton/sodium gradient to generate an additional 2.5 mol of reduced ferredoxin through an RNF complex. This ferredoxin is subsequently used for the reduction of three molecules of CO_2 to 3 mol of CO in the carbonyl branch of the WLP. The WLP carbonyl branch then converts this CO and the three remaining moles of CH_3-THF through acetyl-CoA to acetate, generating 3 mol of ATP (Kremp *et al*., 2018).

Methanol dehydrogenase pathways are organized in three distinct clusters: MxaFI, Mdh2 and XoxF. Both MxaFI and XoxF methanol dehydrogenase clusters occur in a wide range of microorganisms and environments, including oceans, soils, or the human microbiome (Rusch *et al*., 2007; Lidbury *et al*., 2014; Dinasquet *et al*., 2018; Pietzke *et al*., 2020). Mdh2-type is less widespread and has been detected in soil environments only (Kalyuzhnaya *et al*., 2008; Kolb, 2009). Although functionally similar, MxaFI and Mdh2 only share about 35% of amino acid identity, while Mdh2 shares up to 80% identity to other alcohol dehydrogenases with a low affinity for

methanol (Anthony and Williams, 2003; Kalyuzhnaya *et al*., 2008; Lu *et al*., 2012; Keltjens *et al*., 2014; Chistoserdova and Kalyuzhnaya, 2018). MxaFI and Mdh2 methanol dehydrogenases catalyse the conversion of methanol to formaldehyde, releasing two electrons which are then shuttled to cytochrome *c*, whereas XoxF catalyses the conversion of methanol to formate. Mdh2 utilizes NAD(P) as cofactor to shuttle electrons to cytochrome *c* (Zhang *et al*., 2017). Both MxaFI and XoxF enzyme systems utilize pyrroloquinoline quinone (PQQ) as cofactor for electron transport to cytochrome *c*, but they differ in the active site metal. MxaFI utilizes calcium, whereas XoxF utilizes a range of rare earth elements called lanthanides (Picone and Op den Camp, 2019). Incorporation of strontium *in vitro* instead of calcium by MxaFI methanol dehydrogenase has been reported, resulting in increased reaction rates (threefold over calcium) and lower activation energy (by 13.4 kJ mol^{-1}) (Harris and Davidson, 1994). While calcium is abundantly present in seawater, with concentrations measured in the millimolar range, strontium is available with concentrations around 150 μM. Whether all microorganisms containing these methanol dehydrogenases are able to incorporate strontium *in vivo* and whether this has ecological meaning in regards to methanol competitiveness for microorganisms utilizing this strategy requires further research. XoxF enzymes can work with any element of the lanthanide group, although the specific activity is higher with the two lightest lanthanides, lanthanum and cerium, compared with heavier lanthanides like neodymium or promethium (Kiene *et al*., 1986; Keltjens *et al*., 2014; Chistoserdova and Kalyuzhnaya, 2018; Picone and Op den Camp, 2019). While XoxF is mostly described in aerobic marine microorganisms, *Ca*. Methylomirabilis oxyfera utilizes XoxF in its metabolism, which is intracellular aerobic (Ramachandran and Walsh, 2015; Taubert *et al*., 2017; Chistoserdova and Kalyuzhnaya, 2018; Howat *et al*., 2018). Under high calcium and low lanthanide concentrations, the methanotroph *Methylomicrobium buryatense*, which contains both methanol dehydrogenase pathways in its genome, has a higher expression of XoxF than of MxaFI (Chu and Lidstrom, 2016). Interestingly, while metagenomes of anaerobic terrestrial environments revealed multiple XoxF-type alcohol dehydrogenases, PQQ biosynthesis requires molecular oxygen (Velterop *et al*., 1995; Magnusson *et al*., 2004; Martins *et al*., 2019). Diffusion of PQQ from aerobic marine sediments, where XoxF is the most abundant methanol dehydrogenase could provide anoxic sediments with this cofactor (Ramachandran and Walsh, 2015; Taubert *et al*., 2019). Furthermore, it cannot be excluded that alternative, unknown pathways for PQQ biosynthesis that may not involve oxygen exist.

Microbial ecology of methanol degradation in marine environments

The processes mentioned in the previous paragraph occur in many phyla of both *Bacteria* and *Archaea*, including SRM, nitrate-reducing microorganisms, acetogens and methanogens which all occur in marine sediments. Furthermore, metals such as ferric iron or manganese can be present in these environments and can act as an electron acceptor for some methanol oxidizers (Leu *et al*., 2020). However, near neutral pH these compounds are insoluble and occur mainly as minerals, severely limiting their use as electron acceptor without specialized extracellular electron transfer (EET) systems, as found in genera like *Geobacter* or *Shewanella* (Shi *et al*., 2016). Although microorganisms capable of EET utilizing methanol have been described, such as *Shewanella putrefaciens*, there is very little known on their role in marine sediments (Daniel *et al*., 1999). Multiple marine species capable of growing on methanol in anaerobic conditions have been isolated and characterized, and these are described in Table 2.

SRMs occur amongst seven lineages, five of which are within the domain *Bacteria* and two in the domain *Archaea*, although methanol-utilizing SRM have only been described in *Bacteria*. Bacterial SRM belong to Deltaproteobacteria, Clostridia, Nitrospirae, Thermodesulfobacteria and Thermodesulfobiaceae (Muyzer and Stams, 2008). However, based on single-copy marker gene analysis, the phylum Deltaproteobacteria was recently proposed to be divided into the sulfate-reducing phylum Desulfobacterota and the non-sulfate reducing phyla Myxococcota, Bdellovibrionota and SAR324. Additionally, this assessment places the phylum Thermodesulfobacteria within the phylum Desulfobacterota (Waite *et al*., 2020). Furthermore, based on metagenomic datasets, 13 bacterial and archaeal phyla were identified to have the genes for sulfate reduction (*DsrABCD*), thereby doubling the number of known taxa (Anantharaman *et al*., 2018). Only a few marine sulfate reducers have been proven to grow with methanol as the sole electron donor and carbon source (Table 2). However, methanol is not conventionally tested as substrate in the characterization of newly isolated marine SRM.

As the most energetically favourable electron acceptor after oxygen that is abundantly available, nitrate reduction is common at the oxic–anoxic interphase, carried out by anaerobes and facultative anaerobes (Kuypers *et al*., 2018). However, there is a lack of investigation on the use of methanol by nitrate reducers in oxygen minimum zones or marine anoxic sediments. While dissolved organic matter is often not limiting in these systems, methanol is available and yields comparable energy as

Table 2. Isolated marine microorganisms capable of growing on methanol and their growing conditions.

Name organism	Original isolation source	Temperature range (°C)	pH range	Reference
Sulfate-reducing microorganisms				
Desulfallas arcticus	Marine surface sediment, Svalbard, Norway	26–46.5	7.1–7.5	Vandieken *et al*. (2006); Watanabe *et al*. (2018)
Desulfoconvexum algidum	Marine surface sediment, Svalbard, Norway	0–20	7.2–7.4	Konneke *et al*. (2013)
Desulfospira joergensenii[a]	Marine sediment, Arcachon Bay, France	8–30	7.4-ND	Finster *et al*. (1997)
Desulfatiglans anilini	Marine sediment, North Sea coast, Germany	12–40	6–8	Schnell *et al*. (1989)
Desulfosporosinus nitroreducens	Baltic sea sediment,	10–30	6.4–8.1	Vandieken *et al*. (2017)
Nitrate-reducing microorganisms				
Methylophaga nitratireducenticrescens	Seawater denitrification reactor, Montreal Canada	15–37	6–11	Labbé *et al*. (2007)
Acetogens				
Acetobacterium woodii[b]	Oyster pond, Massachusetts	2–45	5.9–8.5	Balch *et al*. (1977)
Methanogens				
Methanococcoides methylutens	Scripps Canyon, California	30–35	7–7.5	Sowers and Ferry, 1983; Watkins *et al*. (2014)
Methanococcoides burtonii	Saltwater lake in Antarctica	5.6–29.5	6.8–8	Franzmann *et al*. (1992); Watkins *et al*. (2014)
Methanococcoides alaskense	Skan Bay, Alaska	−2.3-28.4	6.3–7.5	Singh *et al*. (2005); Watkins *et al*. (2014)
Methanococcoides vulcani	Napoli mud volcano, Mediterranean Sea	ND-35	6–7.8	L'Haridon *et al*. (2014); Watkins *et al*. (2014)
Methanosarcina acetivorans	Scripps Canyon, California	10–50	5.5–8.5	Ferry (1999); Sowers *et al*. (1984)
Methanosarcina baltica	Baltic sea	−22.3-27	4.9–8.5	Von Klein *et al*. (2002)
Methanosarcina semesiae	Dar es Salaam mangrove, Tanzania	30–35	6.5–7.5	Lyimo *et al*. (2009)
Methanosarcina siciliae	Scripps canyon, California	15–40	5–7.5	Elberson and Sowers (1997)
Methermicoccus shengliensis	Shengli oilfield, South China Sea	50–70	5.5–8	Cheng *et al*. (2007)
Methanohalophilus halophilus	Shark Bay, Australia	26–36	6.3–8	Wilharm *et al*. (1991)
Methanolobus bombayensis	Arabian sea	15–43	6.2–8.3	Kadam *et al*. (1994)
Methanolobus vulcani	San Francisco Bay, California	13–45	6–7.5	Kadam and Boone (1995)
Methanolobus profundi	Deep subsurface sediments, Movara, Japan	9–37	6.1–7.8	Mochimaru *et al*. (2009)
Methanolobus taylorii	San Francisco Bay, California	5–45	5.7–9.2	Oremland and Boone (1994)
Methanolobus tindarius	Tindari, Sicily	7–50	5.5–8	König and Stetter (1982)

[a]*D. joergensenii* showed sulfide production on methanol but no growth.
[b]*A. woodii* was isolated from an ocean inlet that was closed off to the sea.

DOM per reducing equivalent (Nurse, 1980). While freshwater denitrification with methanol as electron donor is widely studied due to its biotechnological relevance, whether this process occurs in marine sediments is less clear (Labbé *et al*., 2007; Villeneuve *et al*., 2013).

Acetogens are a diverse group of bacteria, occurring in 22 genera comprising over 100 species (Drake *et al*., 2008). Their key defining trait is the utilization of the Wood–Ljungdahl pathway to fix CO_2 for both energy and biomass production. This metabolism is widely dispersed amongst many habitats, and acetogens are metabolically flexible, incorporating a broad range of electron donors besides C1 compounds, such as sugars or organic acids (Drake *et al*., 2008). Currently, there are few well-characterized marine acetogens capable of growing on methanol (Table 2). *Acetobacterium woodii* was isolated from a former ocean inlet that was closed off but had characteristics similar to the sea (Drake *et al*., 2008; Kremp *et al*., 2018). Furthermore, Schuppert and Schink (1990) isolated an acetogen from the marine Rio Marin in Venice, Italy (Schuppert and Schink, 1990). However, this strain was never deposited in a culture collection. About 67% of all cultivated acetogens have been shown to grow on methanol (Lever *et al*., 2010). Acetogenesis has been described in enriched cultures from marine sediments, and genetic evidence for genes in the Wood–Ljungdahl pathway, such as the presence of formyltetrahydrofolate synthetase and methenyltetrahydrofolate cyclohydrolase coding genes, have been found in metagenomes from a diverse range of marine systems, for example the Juan de Fuca Ridge, the Guaymas Basin, the Baltic Sea and the Arctic Sea

(Lovell and Leaphart, 2005; Lever *et al*., 2010; Kirchman *et al*., 2014; He *et al*., 2016; Beulig *et al*., 2018; Jochum *et al*., 2018; Marshall *et al*., 2018). These findings suggest that acetogenesis and the Wood–Ljungdahl pathway are widely spread in marine systems.

Methanogens were canonically only described within the archaeal phylum *Euryarchaeota*, comprising seven orders and 29 genera (Bapteste *et al*., 2005; Holmes and Smith, 2016). However, recent genomic discoveries have found organisms containing genes coding for methanogenic pathways in the distantly related archaeal phyla *Bathyarchaeota* and *Verstraetearchaeota*, suggesting a much wider phylogenetic range of this metabolism than previously thought (Evans *et al*., 2015; Vanwonterghem *et al*., 2016; Adam *et al*., 2017; Evans *et al*., 2019). Besides marine systems, methanogens have mainly been isolated from animal rumen, rice paddies, soils and freshwater systems (Lyu *et al*., 2018). In marine anaerobic systems, methanogens occur in the sulfate-depleted zone of the sediment, usually several meters below the seafloor, as most methanogens are outcompeted for their common substrates hydrogen and acetate by SRM (Harrison *et al*., 2009). However, methylotrophic methanogenesis has been described to occur in the sulfate zone of the sediment and attributed to the utilization of non-competitive substrates such as methanol (Sela-Adler *et al*., 2017). Furthermore, through interspecies hydrogen transfer, syntrophic interactions between methanogens and SRM for substrates are possible, leading to methane production in the sulfate reduction zone (Dolfing *et al*., 2008; Ozuolmez *et al*., 2015). It is uncertain whether methanol can be utilized in syntrophic interactions in marine environments. A recent study of Mediterranean Sea sediments suggested methanol as being the primary source of methane in sulfate-rich sediments as hydrogenotrophic methanogenesis appeared to be outcompeted by sulfate reduction, with up to 98% of total methane produced in the top sediment deriving from methanol, suggesting methanol to be the main source of methanogenesis (Zhuang *et al*., 2018a,b). Cultured methylotrophic methanogens comprise three orders, *Methanosarcinales*, *Methanobacteriales* and *Methanomassiliicoccales*, although only *Methanosarcinales* have marine representatives. An overview of methanol-utilizing methanogens currently isolated is shown in Table 2. Based on genomic information, methylotrophic methanogenesis has been found outside of the canonical methanogenic phyla, such as *Verstraetearchaeota*, which suggests a much broader phylogeny of methylotrophic methanogenesis than previously thought (Vanwonterghem *et al*., 2016).

Methanol was considered to be a non-competitive substrate for methanogens, explaining the co-occurrence of methanogens in sulfate-rich sediments, where methanogens would be outcompeted by SRM for common substrates such acetate or hydrogen (Oremland and Polcin, 1983). However, in situ oxidation rates of methanol to CO_2 were much higher than solely methanogenesis rates would allow (Zhuang *et al*., 2018a; 2019a,b). Furthermore, radiotracer experiments have shown that in intertidal sediments in Maine, United States, methanol is mostly oxidized through sulfate reduction with methanogenesis only contributing 2.5% of all methanol oxidized (King *et al*., 1983). Coexistence of methanogenesis along with acetogenesis and sulfate reduction is thermodynamically possible, as all methanol conversion reactions have relatively similar Gibbs free energy, i.e. −105, −120 and −133 kJ mol $^{-1}$ for acetogenesis, sulfate reduction and methanogenesis respectively (values calculated for hydrogen partial pressure of maximum 10 nM) (Meulepas *et al*., 2010; Lever, 2011; Lyu *et al*., 2018). Due to the similar energy gains for the different processes, the driving forces for competitive advantage on growth on methanol is the affinity of the metabolic interaction for the substrate and kinetics of the enzymes involved in methanol oxidation, as well as auxotrophy, and the availability of cofactors and catalytic metals in the environment.

It is assumed that microorganisms containing methanol methyltransferase pathways outcompete microorganisms containing methanol dehydrogenases under optimal conditions for both pathways, due to the higher affinity of methyltransferases for methanol (Florencio *et al*., 1993; Florencio *et al*., 1994). This could mean methanogens and acetogens might be able to outcompete SRM for methanol. Several studies on environmental systems have indicated that in anaerobic methanol degradation, cobalt is often the limiting factor (Florencio *et al*., 1993). As cobalt is the active metal in methyltransferases, this corroborates the theory of competitive advantage of methanogens and acetogens. However, Sousa *et al*. (2018) showed the presence of methanol methyltransferase genes in the genome of the sulfate-reducing bacterium *Desulfofundulus kuznetsovii* (formerly *Desulfotomaculum kuznetsovii*) as well as proteomic evidence for an upregulation of the MT system in the presence of cobalt (Sousa *et al*., 2018; Watanabe *et al*., 2018). This would mean that under cobalt-rich conditions, this SRM would be able to compete with methanogens and acetogens for the available methanol. Under cobalt-limiting conditions, it has a competitive advantage as the methanol dehydrogenase is not dependent on cobalt. While *D. kuznetsovii* is not a marine organism, the genes encoding for the methyltransferase pathway in *D. kuznetsovii* have orthologues in many other closely related SRM, for example the marine *Desulfallas arcticus*, *Desulfosporosinus fructosivorans* or the freshwater species *Desulfosporosinus merideii* and *Desulfosporosinus lacus* (Ramamoorthy *et al*., 2006;

Vandieken *et al*., 2006, 2017; Pester *et al*., 2012). Anoxic marine sediments have been found to be relatively rich in cobalt, while it is depleted in the oxic sediment and pore waters above (Heggie and Lewis, 1984). This suggests the possibility of rapid consumption in the oxic–anoxic interphase, indicating competitive advantages for microorganisms containing both pathways.

Metagenomic assessment of key genes involved in methanol metabolism in marine sediments

To assess the dispersion of methanol conversion amongst marine sediments worldwide, we mined core metabolic genes involved in the cycling of methanol in deposited annotated metagenomes in the Integrated Microbial Genomes & Microbiomes (IMG) v.5.0 database following the procedure described by Chen *et al*. (2019). Six genes involved in methanol utilization pathways were selected: three methanol methyltransferase genes: *mtaA; mtaB; mtaC*), one lanthanide-dependent alcohol dehydrogenase (*xoxF*), one methanol-derived formaldehyde dehydrogenase (*fdhA*) and one pectin methanolesterase (*pesT*) (Kanehisa *et al*., 2016). Formaldehyde dehydrogenase was chosen over canonical methanol dehydrogenase due to the high chance of methanol dehydrogenases mistakenly being annotated as other alcohol dehydrogenases, as there is a high similarity between residues between these enzymes and thus increasing the risk of false positives (Reid and Fewson, 1994). Pectin esterase was included to test the hypothesis whether methanol is produced in sediments from pectin.

Utilizing the search function of IMG and manual curation, all metagenomes of anoxic marine sediment samples were exported. In total, 246 metagenomes were selected for this assay. Of these metagenomes, annotation data and metadata containing information on sampling location and sample site water column depth were also collected (Supplementary Table S1). Supplementary Fig. S1 shows a world map with sample locations of all metagenomes grouped by water column height (Fig. S1). Most of the metagenomes derived from datasets close to shores, based on the metadata supplied by IMG (Table S1). This is unsurprising, as deep-sea sampling is logistically difficult and expensive (Clark *et al*., 2016). Likewise, most datasets originate from either the US coasts or the North Sea in Europe, as they are relatively close to large, well-funded marine institutes. Thus most information on methanol cycling as discussed in this review is biased to these environments. There is only a very limited set of metagenomes from deep ocean samples, for example IMG3300010241-3 obtained from the South China Sea or IMG3300016982 obtained from the Southern Atlantic Ocean. Thus, it is difficult to differentiate between coastal ecosystems, which are generally richer in organic material and have a higher carbon turnover than deeper sea ecosystems (Rowe *et al*., 1990; Böer *et al*., 2009).

Using a hidden Markov model –based approach, all metagenomes were scanned for the listed genes. Detailed workflow and tools used can be found in Supplementary Text 1. Total data output was visualized using the R package pheatmap (Kolde, 2019; R Core Team, 2013). Figure 2 displays the results of this metagenome mining effort. Fig. S2a–f shows world maps with corresponding positive locations for each of the six mapped genes.

Based on the prevalence of genes involved in methanol conversion, the utilization is widespread in diverse anoxic marine sediments. Out of 246 published metagenomes, 78% mapped positively for at least one of the selected marker genes in this study. Over 50% of metagenomes mapped positively for at least three selected marker genes, suggesting utilization of methanol by all trophic groups discussed in this review. Interestingly, *mtaB* is much less present than *mtaC*; 117 positive hits in all metagenomes versus 172 for *mtaC*, even though for the utilization of methanol *MtaB* and *mtaC* form a complex (Ferry, 1999). Whether this represents evolutionary artefacts or alternative pathways involving orthologues of these genes is not known. As *MtaC* contains a corrinoid centre to which the methyl group of methanol is bound, it is possible it acts as a carrier for methyl groups derived from non-methanol origins, for example trimethylamine, which is widely present in marine sediments and utilized by methanogens (Ferguson *et al*., 2011; Sun *et al*., 2019). Lanthanide-dependent methanol dehydrogenase could be detected in 112 of the 246 datasets, whereas formaldehyde dehydrogenase was found in 87 datasets. As *xoxF* produces formaldehyde, it is possible other, currently unknown types of formaldehyde incorporation are present in these metagenomes. Furthermore, the presence of *xoxF* as artefacts in these metagenomes that are not actively utilized is also possible. Pectin esterase was present in 52 out of 246 metagenomes. While this was the least present of all studied genes, this still supports the hypothesis that complex carbohydrate degradation can occur in marine sediments, rather than only in the upper water column.

Concluding remarks

Methanol is an important compound in the global biogeochemical cycles, which has received little attention in marine sediments. There are still many gaps in our knowledge on the prevalence of methanol in these environments, how it becomes available and which

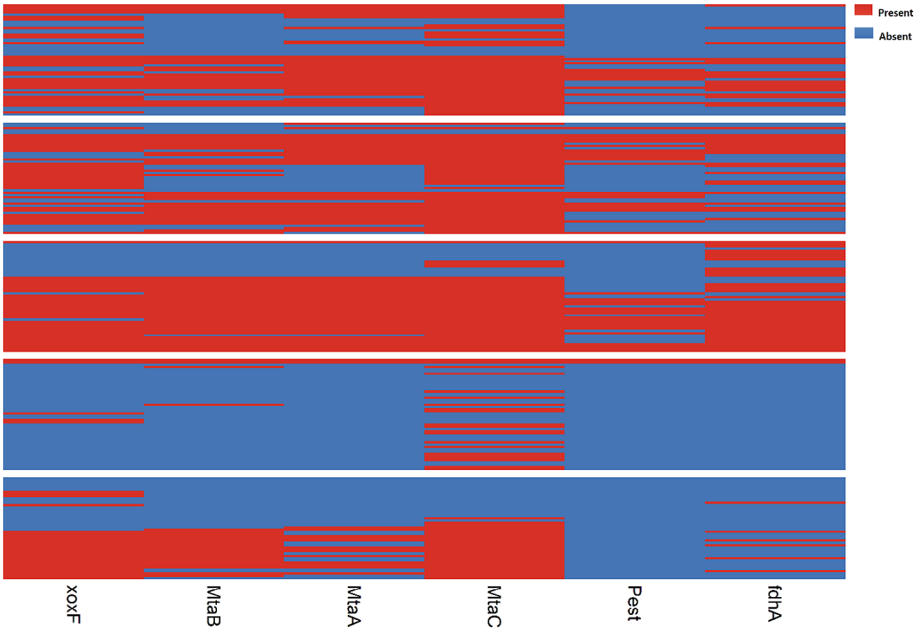

Fig. 2. Metagenomic mining heatmap. Red indicates presence, blue absence. Gene abbreviations: xoxF, lanthanide dependent methanol dehydrogenase F; mtaA, methanol methyltransferase A; mtaB, methanol methyltransferase B; mtaC, methanol methyltransferase C; fdhA, formaldehyde dehydrogenase A; pest, pectin methanolesterase. Each cluster denotes 50 metagenomes screened. [Color figure can be viewed at wileyonlinelibrary.com]

microorganisms are involved in its cycling. The utilization of non-canonical metals in methanol dehydrogenases requires a rethinking of bio-active metals involved in this process. Cultivation approaches fail to recover the vast majority of microorganisms from marine sediments. The use of metagenomic data provides a reliable indication of the diversity and potential functions of specific microorganisms. In this regard, our approach of data mining marine sediment metagenomes for key methanol metabolism genes indicated this compound is released and utilized in anoxic marine sediments worldwide in a variety of ways. Thus, it can be concluded that methanol utilization is an active force in anoxic marine sediments, based on their genomic presence in these environments. This methodology can aid in the further understanding of the ecology of marine anoxic systems.

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Author contributions

P.Q.F.: Conceptualization (supporting), data curation (lead), formal analysis (lead), writing—original draft preparation (lead). I.S.-A.: Writing—review and editing (supporting). A.J.M.S.: Writing—review and editing (supporting), validation (equal), conceptualization (supporting). L.V.: Conceptualization (equal), validation (equal), writing—review and editing (supporting). D.Z.S. Conceptualization (equal), validation (equal), writing—review and editing (lead).

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1. Supporting Information.