





## Minireview

## Anaerobic microbial methanol conversion in marine sediments

Peter Q. Fischer <sup>1,2\*</sup> Irene Sánchez-Andrea <sup>1</sup>,  
Alfons J. M. Stams<sup>1,3</sup> Laura Villanueva <sup>2,4</sup> and  
Diana Z. Sousa <sup>1</sup>

<sup>1</sup>Laboratory of Microbiology, Wageningen University & Research, Stippeneng 4, Wageningen, 6708 WE, The Netherlands.

<sup>2</sup>Department of Marine Microbiology and Biogeochemistry, Royal Netherlands Institute for Sea Research, P.O. Box 59, Den Burg, Texel, 7197 AB, The Netherlands.

<sup>3</sup>Centre of Biological Engineering, University of Minho, Campus de Gualtar, Braga, 4710-057, Portugal.

<sup>4</sup>Faculty of Geosciences, Utrecht University, Princetonlaan 8a, Utrecht, 3584 CB, The Netherlands.

## 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.**

## 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,

Received 3 December, 2020; revised 9 February, 2021; accepted 10 February, 2021. \*For correspondence. E-mail peter.fischer@wur.nl; Tel. +31 317 483 116.

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  $\mu\text{M}$  and over 100  $\mu\text{M}$  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  $\text{CO}_2$  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<sup>-1</sup> 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<sup>-1</sup>, 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<sup>-1</sup> day<sup>-1</sup> (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 <sup>13</sup>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 $\mu\text{M}$ at sea floor 1 $\mu\text{M}$ 100–400 cm bsf 6 $\mu\text{M}$ 500 cm bsf	Zhuang <i>et al.</i> (2014)
Northern Gulf of Mexico	0–30 cm below seafloor	2 $\mu\text{M}$ at sea floor 65 $\mu\text{M}$ 30 cm bsf	Zhuang <i>et al.</i> (2014)
South China Sea	0–700 cm below seafloor	4.3 $\mu\text{M}$ at sea floor 111.7 $\mu\text{M}$ at 700 cm bsf	Zhuang <i>et al.</i> (2019a,b)
Umitaka Spur, eastern Japan Sea	0–35 m below seafloor	0.3–3.5 $\mu\text{M}$ at 0–3 m bsf 20 $\mu\text{M}$ at 30 m bsf	Yanagawa <i>et al.</i> (2016)
Intertidal sediment, Lowes Cove, Maine	Seafloor	0.5–3.5 $\mu\text{M}$	King <i>et al.</i> (1983)
Guaymas Basin, Gulf of California	0–40 cm below seafloor	0.2–2 $\mu\text{M}$ at seafloor 36.7 $\mu\text{M}$ at 35 cm bsf	Zhuang <i>et al.</i> (2019a,b)
Western Mediterranean Sea	0–500 cm below seafloor	0.5–1.5 $\mu\text{M}$ across all depths	Zhuang <i>et al.</i> (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).

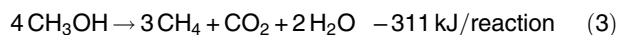
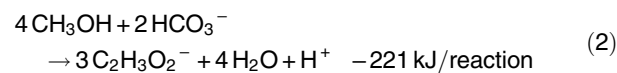
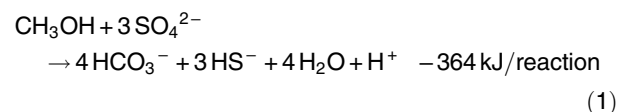
Chemolithotrophic microorganisms present in anaerobic sediments are capable of producing methanol. Anaerobic oxidation of methane occurs in most anoxic systems where methane is present, by methane-oxidizing microorganisms or syntrophic microbial communities capable of using sulfate, nitrate, manganese 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. Methyloirabilis 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).

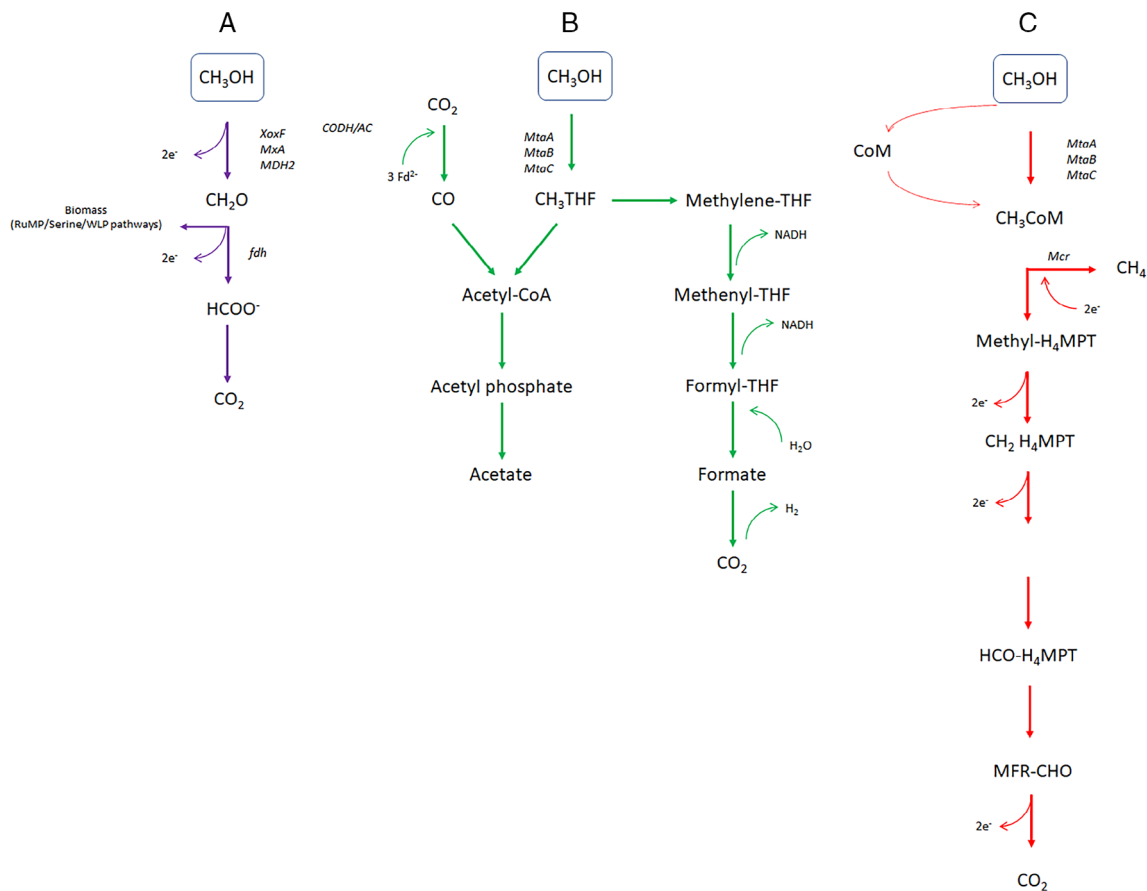
### Biochemistry of microbial methanol utilization

In anaerobic environments, methanol can be converted by three distinct processes: oxidation to CO<sub>2</sub> (e.g. by sulfate-reducing microorganisms (SRM), Equation 1), conversion with CO<sub>2</sub> to acetate (by acetogens, Equation 2), or conversion to methane and CO<sub>2</sub> (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.



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<sub>3</sub>-CoM or CH<sub>3</sub>-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<sub>2</sub> through a reversal of the hydrogen/CO<sub>2</sub> methanogenesis pathway. This process



**Fig. 1.** Methanol degradation pathways as outlined in this review.

A. Respiratory methanol oxidation. Abbreviations: *xoxF*, lanthanide-dependent methanol dehydrogenase; *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<sub>4</sub>MPT*, tetrahydromethanopterin; *Mcr*, methyl-coenzyme M reductase; *MFR*, methanofuran. [Color figure can be viewed at [wileyonlinelibrary.com](http://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<sub>3</sub>. This CoM-CH<sub>3</sub> 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<sub>2</sub> 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<sub>3</sub>-THF can be integrated into the Wood–Ljungdahl pathway (WLP, Fig. 1). One in four molecules of CH<sub>3</sub>-THF is used in a reversal of the methyl branch of the WLP, oxidizing the methylated group to CO<sub>2</sub> and generating 2 mol of NAD(P)H, 1 mol of ATP and 1 mol of H<sub>2</sub>. The generated H<sub>2</sub> is then utilized in a bifurcating mechanism to generate 0.5 mol of reduced ferredoxin (Fd<sup>2-</sup>). 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<sub>2</sub> 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<sub>3</sub>-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<sup>-1</sup>) (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. Methyloirabilis 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 *Methyloicoccus 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 (Kuyper *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
<b>Sulfate-reducing microorganisms</b>				
<i>Desulfallas arcticus</i>	Marine surface sediment, Svalbard, Norway	26–46.5	7.1–7.5	Vandieken <i>et al.</i> (2006); Watanabe <i>et al.</i> (2018)
<i>Desulfoconvexum algidum</i>	Marine surface sediment, Svalbard, Norway	0–20	7.2–7.4	Konneke <i>et al.</i> (2013)
<i>Desulfospira joergensenii</i> <sup>a</sup>	Marine sediment, Arcachon Bay, France	8–30	7.4–ND	Finster <i>et al.</i> (1997)
<i>Desulfatiglang anilini</i>	Marine sediment, North Sea coast, Germany	12–40	6–8	Schnell <i>et al.</i> (1989)
<i>Desulfosporosinus nitroreducens</i>	Baltic sea sediment,	10–30	6.4–8.1	Vandieken <i>et al.</i> (2017)
<b>Nitrate-reducing microorganisms</b>				
<i>Methylophaga nitratireducens</i>	Seawater denitrification reactor, Montreal Canada	15–37	6–11	Labbé <i>et al.</i> (2007)
<b>Acetogens</b>				
<i>Acetobacterium woodii</i> <sup>b</sup>	Oyster pond, Massachusetts	2–45	5.9–8.5	Balch <i>et al.</i> (1977)
<b>Methanogens</b>				
<i>Methanococcoides methylutens</i>	Scripps Canyon, California	30–35	7–7.5	Sowers and Ferry, 1983; Watkins <i>et al.</i> (2014)
<i>Methanococcoides burtonii</i>	Saltwater lake in Antarctica	5.6–29.5	6.8–8	Franzmann <i>et al.</i> (1992); Watkins <i>et al.</i> (2014)
<i>Methanococcoides alaskense</i>	Skan Bay, Alaska	–2.3–28.4	6.3–7.5	Singh <i>et al.</i> (2005); Watkins <i>et al.</i> (2014)
<i>Methanococcoides vulcani</i>	Napoli mud volcano, Mediterranean Sea	ND–35	6–7.8	L'Haridon <i>et al.</i> (2014); Watkins <i>et al.</i> (2014)
<i>Methanosarcina acetivorans</i>	Scripps Canyon, California	10–50	5.5–8.5	Ferry (1999); Sowers <i>et al.</i> (1984)
<i>Methanosarcina baltica</i>	Baltic sea	–22.3–27	4.9–8.5	Von Klein <i>et al.</i> (2002)
<i>Methanosarcina semesiae</i>	Dar es Salaam mangrove, Tanzania	30–35	6.5–7.5	Lyimo <i>et al.</i> (2009)
<i>Methanosarcina siciliae</i>	Scripps canyon, California	15–40	5–7.5	Elbersen and Sowers (1997)
<i>Methermicoccus shengliensis</i>	Shengli oilfield, South China Sea	50–70	5.5–8	Cheng <i>et al.</i> (2007)
<i>Methanohalophilus halophilus</i>	Shark Bay, Australia	26–36	6.3–8	Wilhelm <i>et al.</i> (1991)
<i>Methanolobus bombayensis</i>	Arabian sea	15–43	6.2–8.3	Kadam <i>et al.</i> (1994)
<i>Methanolobus vulcani</i>	San Francisco Bay, California	13–45	6–7.5	Kadam and Boone (1995)
<i>Methanolobus profundus</i>	Deep subsurface sediments, Movara, Japan	9–37	6.1–7.8	Mochimaru <i>et al.</i> (2009)
<i>Methanolobus taylorii</i>	San Francisco Bay, California	5–45	5.7–9.2	Oremland and Boone (1994)
<i>Methanolobus tindarius</i>	Tindari, Sicily	7–50	5.5–8	König and Stetter (1982)

<sup>a</sup>*D. joergensenii* showed sulfide production on methanol but no growth.

<sup>b</sup>*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<sub>2</sub> 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, methylo-trophic 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 methylo-trophic 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, methylo-trophic methanogenesis has been found outside of the canonical methanogenic phyla, such as *Verstraetearchaeota*, which suggests a much broader phylogeny of methylo-trophic 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<sub>2</sub> 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<sup>-1</sup> 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 *Desulfallus 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).

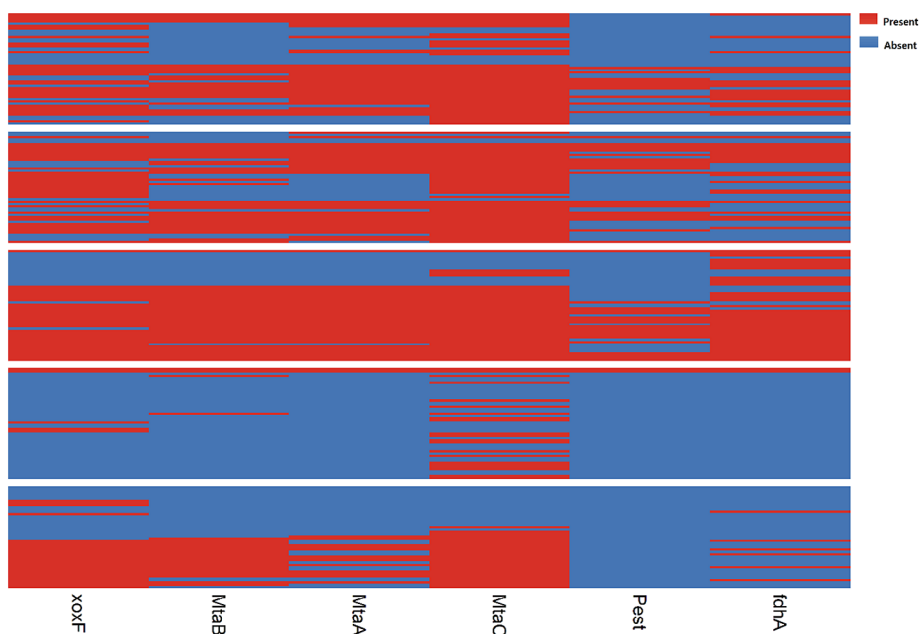
Using a hidden Markov model –based approach, all metagenomes were scanned for the listed genes. Detailed workflow and tools used can be found in Supplementary Text 1. Total data output was visualized using the R package pheatmap (Kolde, 2019; R Core Team, 2013). Figure 2 displays the results of this 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](http://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.

### Acknowledgements

The authors were financially supported by the Gravitation grant (project 024.002.002) of the Netherlands Ministry of Education, Culture and Science and the Netherlands Science Foundation (NWO). The members of the MIB-ETE thesis ring are thanked for fruitful discussions.

### 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).

### References

- Adam, P.S., Borrel, G., Brochier-Armanet, C., and Gribaldo, S. (2017) The growing tree of archaea: new perspectives on their diversity, evolution and ecology. *ISME J* **11**: 2407–2425. <https://doi.org/10.1038/ismej.2017.122>.
- Anantharaman, K., Hausmann, B., Jungbluth, S.P., Kantor, R.S., Lavy, A., Warren, L.A., et al. (2018) Expanded diversity of microbial groups that shape the dissimilatory sulfur cycle. *ISME J* **12**: 1715–1728. <https://doi.org/10.1038/s41396-018-0078-0>.
- Anthony, C., and Williams, P. (2003) The structure and mechanism of methanol dehydrogenase. *Biochim Biophys Acta* **1647**: 18–23. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/12686102>.
- Balch, W.E., Schobert, S., Tanner, R.S., and Wolfe, R.S. (1977) *Acetobacterium*, a new genus of hydrogen-oxidizing, carbon dioxide-reducing, anaerobic bacteria. *Int J Syst Bacteriol* **27**: 355–361. <https://doi.org/10.1099/00207713-27-4-355>.
- Bapteste, E., Brochier, C., and Boucher, Y. (2005) Higher-level classification of the Archaea: evolution of methanogenesis and methanogens. *Archaea* **1**: 353–363. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/15876569>.
- Beale, R., Dixon, J.L., Arnold, S.R., Liss, P.S., and Nightingale, P.D. (2013) Methanol, acetaldehyde, and acetone in the surface waters of the Atlantic Ocean. *J Geophys Res Oceans* **118**: 5412–5425. <https://doi.org/10.1002/jgrc.20322>.
- Beulig, F., Røy, H., Mcglynn, S.E., and Jørgensen, B.B. (2018) Cryptic CH<sub>4</sub> cycling in the sulfate and methane

- transition of marine sediments apparently mediated by ANME-1 archaea. *ISME J* **13**: 250–262. <https://doi.org/10.1038/s41396-018-0273-z>.
- Böer, S.I., Amosti, C., Van Beusekom, J.E.E., and Boetius, A. (2009) Temporal variations in microbial activities and carbon turnover in subtidal sandy sediments. *Biogeosciences* **6**: 1149–1165. <https://doi.org/10.5194/bg-6-1149-2009>.
- Chen, I.M.A., Chu, K., Palaniappan, K., Pillay, M., Ratner, A., Huang, J., *et al.* (2019) IMG/M v.5.0: an integrated data management and comparative analysis system for microbial genomes and microbiomes. *Nucleic Acids Res* **47**: D666–D677. <https://doi.org/10.1093/nar/gky901>.
- Cheng, L., Qiu, T.-L., Yin, X.-B., Wu, X.-L., Hu, G.-Q., Deng, Y., and Zhang, H. (2007) *Methermicoccus shengliensis* gen. nov., sp. nov., a thermophilic, methylophilic methanogen isolated from oil-production water, and proposal of *Methermicoccaceae* fam. nov. *Int J Syst Evol Microbiol* **57**: 2964–2969. <https://doi.org/10.1099/ijs.0.65049-0>.
- Chistoserdova, L. (2015) Methylophilic in natural habitats: current insights through metagenomics. *Appl Microbiol Biotechnol* **99**: 5763–5779. <https://doi.org/10.1007/s00253-015-6713-z>.
- Chistoserdova, L., and Kalyuzhnaya, M.G. (2018) Current trends in methylophilicity. *Trends Microbiol* **26**: 703–714. <https://doi.org/10.1016/j.tim.2018.01.011>.
- Chu, F., and Lidstrom, M.E. (2016) XoxF acts as the predominant methanol dehydrogenase in the type I methanotroph *Methylomicrobium buryatense*. *J Bacteriol* **198**: 1317–1325. <https://doi.org/10.1128/JB.00959-15>.
- Cicerone, R.J., and Oremland, R.S. (1988) Biogeochemical aspects of atmospheric methane. *Global Biogeochem Cycles* **2**: 299–327. <https://doi.org/10.1029/GB0021004p00299>.
- Clark, M.R., Consalvey, M., and Rowden, A.A. (2016) Biological sampling in the deep sea. In *Biological Sampling in the Deep Sea*, Clark, M.R., Consalvey, M., and Rowden, A.A. (eds). Hoboken: John Wiley & Sons. <https://doi.org/10.1002/9781118332535>.
- Cloern, J.E., Foster, S.Q., and Kleckner, A.E. (2014) Phytoplankton primary production in the world's estuarine-coastal ecosystems. *Biogeosciences* **11**: 2477–2501. <https://doi.org/10.5194/bg-11-2477-2014>.
- Costa, K.C., and Leigh, J.A. (2014) Metabolic versatility in methanogens. *Curr Opin Biotechnol* **29**: 70–75. <https://doi.org/10.1016/j.copbio.2014.02.012>.
- Daas, P.J.H., Hagen, W.R., Keltjens, J.T., Van Der Drift, C., and Vogels, G.D. (1996) Activation mechanism of methanol:5-Hydroxybenzimidazolylcobamide methyltransferase from *Methanosarcina barkeri*. *J Biol Chem* **271**: 22346–22351. <https://doi.org/10.1074/jbc.271.37.22346>.
- Daniel, R., Warnecke, F., Potekhina, J.S., and Gottschalk, G. (1999) Identification of the syntrophic partners in a coculture coupling anaerobic methanol oxidation to Fe(III) reduction. *FEMS Microbiol Lett* **180**: 197–203. <https://doi.org/10.1111/j.1574-6968.1999.tb08796.x>.
- D'hondt, S., Inagaki, F., Zarikian, C.A., Abrams, L.J., Dubois, N., Engelhardt, T., *et al.* (2015) Presence of oxygen and aerobic communities from sea floor to basement in deep-sea sediments. *Nat Geosci* **8**: 299–304. <https://doi.org/10.1038/ngeo2387>.
- Dinasquet, J., Tiirola, M., and Azam, F. (2018) Enrichment of bacterioplankton able to utilize one-carbon and methylated compounds in the coastal Pacific Ocean. *Front Mar Sci* **5**: 307. <https://doi.org/10.3389/fmars.2018.00307>.
- Dixon, J.L., Beale, R., and Nightingale, P.D. (2011a) Rapid biological oxidation of methanol in the tropical Atlantic: significance as a microbial carbon source. *Biogeosciences* **8**: 2707–2716. <https://doi.org/10.5194/bg-8-2707-2011>.
- Dixon, J.L., and Nightingale, P.D. (2012) Fine-scale variability in methanol uptake and oxidation: from the microlayer to 1000 m. *Biogeosciences* **9**: 2961–2972. <https://doi.org/10.5194/bg-9-2961-2012>.
- Dixon, J.L., Beale, R., and Nightingale, P.D. (2011b) Microbial methanol uptake in Northeast Atlantic waters. *ISME J* **5**: 704–716. <https://doi.org/10.1038/ismej.2010.169>.
- Dixon, J.L., Beale, R., and Nightingale, P.D. (2013) Production of methanol, acetaldehyde, and acetone in the Atlantic Ocean. *Geophys Res Lett* **40**: 4700–4705. <https://doi.org/10.1002/grl.50922>.
- Dolfing, J., Jiang, B., Henstra, A.M., Stams, A.J.M., and Plugge, C.M. (2008) Syntrophic growth on formate: a new microbial niche in anoxic environments. *Appl Environ Microbiol* **74**: 6126–6131. <https://doi.org/10.1128/AEM.01428-08>.
- Drake, H.L., Gößner, A.S., and Daniel, S.L. (2008) Old Acetogens, new light. *Ann N Y Acad Sci* **1125**: 100–128. <https://doi.org/10.1196/annals.1419.016>.
- Elbersson, M.A., and Sowers, K.R. (1997) Isolation of an acetoclastic strain of *Methanosarcina siciliae* from marine canyon sediments and emendation of the species description for *Methanosarcina siciliae*. *Int J Syst Bacteriol* **47**: 1258–1261. <https://doi.org/10.1099/00207713-47-4-1258>.
- Ettwig, K.F., van Alen, T., van de Pas-Schoonen, K.T., Jetten, M.S.M., and Strous, M. (2009) Enrichment and molecular detection of denitrifying methanotrophic bacteria of the NC10 phylum. *Appl Environ Microbiol* **75**: 3656–3662. <https://doi.org/10.1128/AEM.00067-09>.
- Evans, P.N., Boyd, J.A., Leu, A.O., Woodcroft, B.J., Parks, D.H., Hugenholtz, P., and Tyson, G.W. (2019) An evolving view of methane metabolism in the Archaea. *Nat Rev Microbiol* **1**: 219. <https://doi.org/10.1038/s41579-018-0136-7>.
- Evans, P.N., Parks, D.H., Chadwick, G.L., Robbins, S.J., Orphan, V.J., Golding, S.D., and Tyson, G.W. (2015) Methane metabolism in the archaeal phylum Bathyarchaeota revealed by genome-centric metagenomics. *Science* **350**: 434–438. <https://doi.org/10.1126/science.aac7745>.
- Ferguson, D.J., Longstaff, D.G., and Krzycki, J.A. (2011) Assay of methylophilic methyltransferases from methanogenic archaea. *Methods Enzymol* **494**: 139–158. <https://doi.org/10.1016/B978-0-12-385112-3.00008-1>.
- Ferry, J.G. (1999) Enzymology of one-carbon metabolism in methanogenic pathways. *FEMS Microbiol Rev* **23**: 13–38. <https://doi.org/10.1111/j.1574-6976.1999.tb00390.x>.
- Finster, K., Liesack, W., and Tindall, B.J. (1997) *Desulfospira joergensenii*, gen. nov., sp. nov., a new sulfate-reducing bacterium isolated from marine surface sediment. *Syst*

- Appl Microbiol* **20**: 201–208. [https://doi.org/10.1016/S0723-2020\(97\)80066-5](https://doi.org/10.1016/S0723-2020(97)80066-5).
- Florencio, L., Field, J.A., and Lettinga, G. (1994) Importance of cobalt for individual trophic groups in an anaerobic methanol-degrading consortium. *Appl Environ Microbiol* **60**: 227–234. Retrieved from. <http://www.ncbi.nlm.nih.gov/pubmed/8117078>.
- Florencio, L., Jeniček, P., Field, J.A., and Lettinga, G. (1993) Effect of cobalt on the anaerobic degradation of methanol. *J Ferment Bioeng* **75**: 368–374. [https://doi.org/10.1016/0922-338X\(93\)90136-V](https://doi.org/10.1016/0922-338X(93)90136-V).
- Franzmann, P.D., Springer, N., Ludwig, W., Conway De Macario, E., and Rohde, M. (1992) A methanogenic archaeon from ace Lake, Antarctica: *Methanococoides burtonii* sp. nov. *Syst Appl Microbiol* **15**: 573–581. [https://doi.org/10.1016/S0723-2020\(11\)80117-7](https://doi.org/10.1016/S0723-2020(11)80117-7).
- Galbally, I.E., and Kirstine, W. (2002) The production of methanol by flowering plants and the global cycle of methanol. *J Atmos Chem* **43**: 195–229. <https://doi.org/10.1023/A:1020684815474>.
- Glud, R.N. (2008) Oxygen dynamics of marine sediments. *Mar Biol Res* **4**: 243–289. <https://doi.org/10.1080/17451000801888726>.
- Goorissen, H.P., Stams, A.J.M., and Hansen, T.A. (2004) Methanol utilization in defined mixed cultures of thermophilic anaerobes in the presence of sulfate. *FEMS Microbiol Ecol* **49**: 489–494. <https://doi.org/10.1016/j.femsec.2004.05.004>.
- Gottschalk, G., and Thauer, R.K. (2001) The Na<sup>+</sup>-translocating methyltransferase complex from methanogenic archaea. *Biochim Biophys Acta (BBA) - Bioenergetics* **1505**: 28–36. [https://doi.org/10.1016/S0005-2728\(00\)00274-7](https://doi.org/10.1016/S0005-2728(00)00274-7).
- Grinsven, S., Sinninghe Damsté, J.S., Abdala Asbun, A., Engemann, J.C., Harrison, J., and Villanueva, L. (2020) Methane oxidation in anoxic lake water stimulated by nitrate and sulfate addition. *Environ Microbiol* **22**: 766–782. <https://doi.org/10.1111/1462-2920.14886>.
- Haroon, M.F., Hu, S., Shi, Y., Imelfort, M., Keller, J., Hugenholtz, P., et al. (2013) Anaerobic oxidation of methane coupled to nitrate reduction in a novel archaeal lineage. *Nature* **500**: 567–570. <https://doi.org/10.1038/nature12375>.
- Harris, T.K., and Davidson, V.L. (1994) Replacement of enzyme-bound calcium with strontium alters the kinetic properties of methanol dehydrogenase. *Biochem J* **300**: 175–182. <https://doi.org/10.1042/bj3000175>.
- Harrison, B.K., Zhang, H., Berelson, W., and Orphan, V.J. (2009) Variations in archaeal and bacterial diversity associated with the sulfate-methane transition zone in continental margin sediments (Santa Barbara Basin, California). *Appl Environ Microbiol* **75**: 1487–1499. <https://doi.org/10.1128/AEM.01812-08>.
- He, Y., Li, M., Perumal, V., Feng, X., Fang, J., Xie, J., et al. (2016) Genomic and enzymatic evidence for acetogenesis among multiple lineages of the archaeal phylum Bathyarchaeota widespread in marine sediments. *Nat Microbiol* **16035**: 16035. <https://doi.org/10.1038/NMICROBIOL.2016.35>.
- He, Z., Geng, S., Cai, C., Liu, S., Liu, Y., Pan, Y., et al. (2015) Anaerobic oxidation of methane coupled to nitrite reduction by halophilic marine NC10 bacteria. *Appl Environ Microbiol* **81**: 5538–5545. <https://doi.org/10.1128/AEM.00984-15>.
- Heggie, D., and Lewis, T. (1984) Cobalt in pore waters of marine sediments. *Nature* **311**: 453–455. <https://doi.org/10.1038/311453a0>.
- Heikes, B.G., Chang, W., Pilson, M.E.Q., Swift, E., Singh, H. B., Guenther, A., et al. (2002) Atmospheric methanol budget and ocean implication. *Glob Biogeochem Cycles* **16**: 80–1–80–13. <https://doi.org/10.1029/2002GB001895>.
- Holmes, D.E., and Smith, J.A. (2016) Biologically produced methane as a renewable energy source. *Adv Appl Microbiol* **97**: 1–61. <https://doi.org/10.1016/BS.AAMBS.2016.09.001>.
- Howat, A.M., Vollmers, J., Taubert, M., Grob, C., Dixon, J.L., Todd, J.D., et al. (2018) Comparative genomics and mutational analysis reveals a novel XoxF-utilizing methylotroph in the Roseobacter group isolated from the marine environment. *Front Microbiol* **9**: 766. <https://doi.org/10.3389/fmicb.2018.00766>.
- Jacob, D.J., Field, B.D., Li, Q., Blake, D.R., de Gouw, J., Warneke, C., et al. (2005) Global budget of methanol: constraints from atmospheric observations. *J Geophys Res* **110**: D08303. <https://doi.org/10.1029/2004JD005172>.
- Jochum, L.M., Schreiber, L., Marshall, I.P.G., Jørgensen, B. B., Schramm, A., and Kjeldsen, K.U. (2018) Single-cell genomics reveals a diverse metabolic potential of uncultivated Desulfatiglans-related Deltaproteobacteria widely distributed in marine sediment. *Front Microbiol* **9**: 2038. <https://doi.org/10.3389/fmicb.2018.02038>.
- Jørgensen, B.B., and Boetius, A. (2007) Feast and famine - microbial life in the deep-sea bed. *Nat Rev Microbiol* **5**: 770–781. <https://doi.org/10.1038/nrmicro1745>.
- Kadam, P.C., and Boone, D.R. (1995) Physiological characterization and emended description of *Methanobolus vulcani*. *Int J Syst Bacteriol* **45**: 400–402. <https://doi.org/10.1099/00207713-45-2-400>.
- Kadam, P.C., Ranade, D.R., Mandelco, L., and Boone, D.R. (1994) Isolation and characterization of *Methanobolus bombayensis* sp. nov., a methylotrophic methanogen that requires high concentrations of divalent cations. *Int J Syst Bacteriol* **44**: 603–607. <https://doi.org/10.1099/00207713-44-4-603>.
- Kalyuzhnaya, M.G., Hristova, K.R., Lidstrom, M.E., and Chistoserdova, L. (2008) Characterization of a novel methanol dehydrogenase in representatives of Burkholderiales: implications for environmental detection of methylotrophy and evidence for convergent evolution. *J Bacteriol* **190**: 3817–3823. <https://doi.org/10.1128/JB.00180-08>.
- Kameyama, S., Tanimoto, H., Inomata, S., Tsunogai, U., Ooki, A., Takeda, S., et al. (2010) High-resolution measurement of multiple volatile organic compounds dissolved in seawater using equilibrium inlet-proton transfer reaction-mass spectrometry (EI-PTR-MS). *Mar Chem* **122**: 59–73. <https://doi.org/10.1016/J.MARCHEM.2010.08.003>.
- Kanehisa, M., Sato, Y., Kawashima, M., Furumichi, M., and Tanabe, M. (2016) KEGG as a reference resource for gene and protein annotation. *Nucleic Acids Res* **44**: D457–D462. <https://doi.org/10.1093/nar/gkv1070>.
- Keltjens, J.T., Pol, A., Reimann, J., and Op den Camp, H.J. M. (2014) PQQ-dependent methanol dehydrogenases:

- rare-earth elements make a difference. *Appl Microbiol Biotechnol* **98**: 6163–6183. <https://doi.org/10.1007/s00253-014-5766-8>.
- Kiene, R.P., Catena, A., Miller, L.G., Oremland, R.S., and Capone, D.G. (1986) Metabolism of reduced methylated sulfur compounds in anaerobic sediments and by a pure culture of an estuarine methanogens. *Appl Environ Microbiol* **52**: 1037–1045.
- King, G.M., Klug, M.J., and Lovley, D.R. (1983) Metabolism of acetate, methanol, and methylated amines in intertidal sediments of Lowes Cove, Maine. *Appl Environ Microbiol* **45**(6): 1848–1853. <https://doi.org/10.1128/AEM.45.6.1848-1853.1983>.
- Kirchman, D.L., Hanson, T.E., Cottrell, M.T., and Hamdan, L. J. (2014) Metagenomic analysis of organic matter degradation in methane-rich Arctic Ocean sediments. *Limnol Oceanogr* **59**: 548–559. <https://doi.org/10.4319/lo.2014.59.2.0548>.
- Kolb, S. (2009) Aerobic methanol-oxidizing bacteria in soil. *FEMS Microbiol Lett* **300**: 1–10. <https://doi.org/10.1111/j.1574-6968.2009.01681.x>.
- Kolde, R. (2019). *Phetamap: pretty heatmaps*. Retrieved from <https://cran.r-project.org/web/packages/phetamap/index.html>
- König, H., and Stetter, K.O. (1982) Isolation and characterization of *Methanobacterium tindarius*, sp. nov., a coccoid methanogen growing only on methanol and methylamines. *Zentralbl Bakteriell Mikrobiol Hygiene: I Abt Orig C* **3**: 478–490. [https://doi.org/10.1016/S0721-9571\(82\)80005-7](https://doi.org/10.1016/S0721-9571(82)80005-7).
- Konneke, M., Kuever, J., Galushko, A., and Jorgensen, B.B. (2013) *Desulfoconvexum algidum* gen. nov., sp. nov., a psychrophilic sulfate-reducing bacterium isolated from a permanently cold marine sediment. *Int J Syst Evol Microbiol* **63**: 959–964. <https://doi.org/10.1099/ijs.0.043703-0>.
- Kremp, F., Poehlein, A., Daniel, R., and Müller, V. (2018) Methanol metabolism in the acetogenic bacterium *Acetobacterium woodii*. *Environ Microbiol* **20**: 4369–4384. <https://doi.org/10.1111/1462-2920.14356>.
- Kuypers, M.M.M., Marchant, H.K., and Kartal, B. (2018) The microbial nitrogen-cycling network. *Nat Rev Microbiol* **16**: 263–276. <https://doi.org/10.1038/nrmicro.2018.9>.
- L'Haridon, S., Chalopin, M., Colombo, D., and Toffin, L. (2014) *Methanococcoides vulcani* sp. nov., a marine methylophilic methanogen that uses betaine, choline and N,N-dimethylethanolamine for methanogenesis, isolated from a mud volcano, and emended description of the genus *Methanococcoides*. *Int J Syst Evol Microbiol* **64**: 1978–1983. <https://doi.org/10.1099/ijs.0.058289-0>.
- Labbé, N., Laurin, V., Juteau, P., Parent, S., and Villemur, R. (2007) Microbiological community structure of the biofilm of a methanol-fed, marine denitrification system, and identification of the methanol-utilizing microorganisms. *Microb Ecol* **53**: 621–630. <https://doi.org/10.1007/s00248-006-9168-z>.
- Lang, K., Schuldes, J.J., Klingl, A., Poehlein, A., Daniel, R., and Brune, A. (2015) New mode of energy metabolism in the seventh order of methanogens as revealed by comparative genome analysis of “*Candidatus Methanoplasma termitum*”. *Appl Environ Microbiol* **81**: 1338–1352. <https://doi.org/10.1128/AEM.03389-14>.
- Leu, A.O., Cai, C., McIlroy, S.J., Southam, G., Orphan, V.J., Yuan, Z., et al. (2020) Anaerobic methane oxidation coupled to manganese reduction by members of the Methanoperedenaceae. *ISME J* **14**: 1030–1041. <https://doi.org/10.1038/s41396-020-0590-x>.
- Lever, M.A., Alperin, M.J., Teske, A., Heuer, V.B., Schmidt, F., Hinrichs, K.U., et al. (2010) Acetogenesis in deep seafloor sediments of the Juan de Fuca ridge flank: a synthesis of geochemical, thermodynamic, and gene-based evidence. *Geomicrobiol J* **27**: 183. <https://doi.org/10.1080/01490450903456681>.
- Lever, M.A. (2011) Acetogenesis in the energy-starved deep biosphere - a paradox? *Front Microbiol* **2**: 284. <https://doi.org/10.3389/fmicb.2011.00284>.
- Lidbury, I., Mausz, M.A., Scanlan, D.J., and Chen, Y. (2017) Identification of dimethylamine monooxygenase in marine bacteria reveals a metabolic bottleneck in the methylated amine degradation pathway. *ISME J* **11**: 1592–1601. <https://doi.org/10.1038/ismej.2017.31>.
- Lidbury, I., Murrell, J.C., and Chen, Y. (2014) Trimethylamine N-oxide metabolism by abundant marine heterotrophic bacteria. *Proc Natl Acad Sci U S A* **111**: 2710–2715. <https://doi.org/10.1073/pnas.1317834111>.
- Lovell, C.R., and Leaphart, A.B. (2005) Community-level analysis: key genes of CO<sub>2</sub>-reductive acetogenesis. *Methods Enzymol* **397**: 454–469. [https://doi.org/10.1016/S0076-6879\(05\)97028-6](https://doi.org/10.1016/S0076-6879(05)97028-6).
- Lu, H., Kalyuzhnaya, M., and Chandran, K. (2012) Comparative proteomic analysis reveals insights into anoxic growth of *Methyloversatilis universalis* FAM5 on methanol and ethanol. *Environ Microbiol* **14**: 2935–2945. <https://doi.org/10.1111/j.1462-2920.2012.02857.x>.
- Lyimo, T.J., Pol, A., Jetten, M.S.M., and Op den Camp, H.J. M. (2009) Diversity of methanogenic archaea in a mangrove sediment and isolation of a new *Methanococcoides* strain. *FEMS Microbiol Lett* **291**: 247–253. <https://doi.org/10.1111/j.1574-6968.2008.01464.x>.
- Lyu, Z., Shao, N., Akinyemi, T., and Whitman, W.B. (2018) Methanogenesis. *Curr Biol* **28**: R727–R732. <https://doi.org/10.1016/j.cub.2018.05.021>.
- Magnusson, O.T., Toyama, H., Saeki, M., Rojas, A., Reed, J.C., Liddington, R.C., et al. (2004) Quinone biogenesis: structure and mechanism of PqqC, the final catalyst in the production of pyrroloquinoline quinone. *Proc Natl Acad Sci U S A* **101**: 7913–7918. <https://doi.org/10.1073/pnas.0402640101>.
- Marshall, I.P.G., Karst, S.M., Nielsen, P.H., and Jørgensen, B.B. (2018) Metagenomes from deep Baltic Sea sediments reveal how past and present environmental conditions determine microbial community composition. *Mar Genomics* **37**: 58–68. <https://doi.org/10.1016/J.MARGEN.2017.08.004>.
- Martins P.D., Frank, J., Mitchell, H., Markillie, L.M., and Wilkins, M.J. (2019). Wetland sediments host diverse microbial taxa capable of cycling alcohols. *Appl Environ Microbiol*, **85**. <http://dx.doi.org/10.1128/aem.00189-19>.
- McGlynn, S.E. (2017) Energy metabolism during anaerobic methane oxidation in ANME archaea. *Microbes Environ* **32**: 5–13. <https://doi.org/10.1264/jisme2.ME16166>.
- Meulepas, R.J.W., Jagersma, C.G., Khadem, A.F., Stams, A.J.M., and Lens, P.N.L. (2010) Effect of

- methanogenic substrates on anaerobic oxidation of methane and sulfate reduction by an anaerobic methanotrophic enrichment. *Appl Microbiol Biotechnol* **87**: 1499–1506. <https://doi.org/10.1007/s00253-010-2597-0>.
- Millet, D.B., Jacob, D.J., Custer, T.G., De Gouw, J.A., Goldstein, A.H., Karl, T., et al. (2008) Atmospheric chemistry and physics new constraints on terrestrial and oceanic sources of atmospheric methanol. *Atmos Chem Phys* **8**: 6887.
- Mincer, T.J., and Aicher, A.C. (2016) Methanol production by a broad phylogenetic array of marine phytoplankton. *PLoS One* **11**: e0150820. <https://doi.org/10.1371/journal.pone.0150820>.
- Mochimaru, H., Tamaki, H., Hanada, S., Imachi, H., Nakamura, K., Sakata, S., and Kamagata, Y. (2009) *Methanobus profundus* sp. nov., a methylotrophic methanogen isolated from deep subsurface sediments in a natural gas field. *Int J Syst Evol Microbiol* **59**: 714–718. <https://doi.org/10.1099/ijs.0.001677-0>.
- Muzyer, G., and Stams, A.J.M. (2008) The ecology and biotechnology of sulphate-reducing bacteria. *Nat Rev Microbiol* **6**: 441–454. <https://doi.org/10.1038/nrmicro1892>.
- Neufeld, J.D., Boden, R., Moussard, H., Schäfer, H., and Murrell, J.C. (2008) Substrate-specific clades of active marine methylotrophs associated with a phytoplankton bloom in a temperate coastal environment. *Appl Environ Microbiol* **74**: 7321–7328. <https://doi.org/10.1128/AEM.01266-08>.
- Nobu, M.K., Narihiro, T., Kuroda, K., Mei, R., and Liu, W.-T. (2016) Chasing the elusive Euryarchaeota class WSA2: genomes reveal a uniquely fastidious methyl-reducing methanogen. *ISME J* **10**: 2478–2487. <https://doi.org/10.1038/ismej.2016.33>.
- Nurse, G.R. (1980) Denitrification with methanol: microbiology and biochemistry. *Water Res* **14**: 531–537. [https://doi.org/10.1016/0043-1354\(80\)90221-3](https://doi.org/10.1016/0043-1354(80)90221-3).
- Oremland, R.S., and Boone, D.R. (1994) *Methanobus taylorii* sp. nov., a new methylotrophic, estuarine methanogen. *Int J Syst Bacteriol* **44**: 573.
- Oremland, R.S., and Culbertson, C.W. (1992) Importance of methane-oxidizing bacteria in the methane budget as revealed by the use of a specific inhibitor. *Nature* **356**: 421–423. <https://doi.org/10.1038/356421a0>.
- Oremland, R.S., and Polcin, S. (1983) Methanogenesis and sulfate reduction: competitive and noncompetitive substrates in estuarine sediments. *Deep Sea Res Part B Oceanogr Lit Rev* **30**: 470. [https://doi.org/10.1016/0198-0254\(83\)90262-5](https://doi.org/10.1016/0198-0254(83)90262-5).
- Ozuolmez, D., Na, H., Lever, M.A., Kjeldsen, K.U., Jørgensen, B.B., and Plugge, C.M. (2015) Methanogenic archaea and sulfate reducing bacteria co-cultured on acetate: teamwork or coexistence? *Front Microbiol* **6**: 1–12. <https://doi.org/10.3389/fmicb.2015.00492>.
- Padilla, C.C., Bristow, L.A., Sarode, N., Garcia-Robledo, E., Gómez Ramírez, E., Benson, C.R., et al. (2016) NC10 bacteria in marine oxygen minimum zones. *ISME J* **10**: 2067–2071. <https://doi.org/10.1038/ismej.2015.262>.
- Pester, M., Brambilla, E., Alazard, D., Rattei, T., Weinmaier, T., Han, J., et al. (2012) Complete genome sequences of *Desulfosporosinus orientis* DSM765T, *Desulfosporosinus youngiae* DSM17734T, *Desulfosporosinus meridiei* DSM13257T, and *Desulfosporosinus acidiphilus* DSM22704T. *J Bacteriol* **194**: 6300–6301. <https://doi.org/10.1128/JB.01392-12>.
- Picone, N., and Op den Camp, H.J. (2019) Role of rare earth elements in methanol oxidation. *Curr Opin Chem Biol* **49**: 39–44. <https://doi.org/10.1016/J.CBPA.2018.09.019>.
- Pietzke, M., Meiser, J., and Vazquez, A. (2020) Formate metabolism in health and disease. *Mol Metab* **33**: 23–37. <https://doi.org/10.1016/j.molmet.2019.05.012>.
- R Core Team. (2013). R: A language and environment for statistical computing. *R Foundation for Statistical Computing*. Vienna, Austria. <http://www.R-project.org/>.
- Ramachandran, A., and Walsh, D.A. (2015) Investigation of XoxF methanol dehydrogenases reveals new methylotrophic bacteria in pelagic marine and freshwater ecosystems. *FEMS Microbiol Ecol* **91**: fiv105. <https://doi.org/10.1093/femsec/fiv105>.
- Ramamoorthy, S., Sass, H., Langner, H., Schumann, P., Kroppenstedt, R.M., Spring, S., et al. (2006) *Desulfosporosinus lacus* sp. nov., a sulfate-reducing bacterium isolated from pristine freshwater lake sediments. *Int J Syst Evol Microbiol* **56**: 2729–2736. <https://doi.org/10.1099/ijs.0.63610-0>.
- Read, K.A., Carpenter, L.J., Arnold, S.R., Beale, R., Nightingale, P.D., Hopkins, J.R., et al. (2012) Multiannual observations of acetone, methanol, and acetaldehyde in remote tropical Atlantic air: implications for atmospheric OVOC budgets and oxidative capacity. *Environ Sci Technol* **46**: 11028–11039. <https://doi.org/10.1021/es302082p>.
- Reid, M.F., and Fewson, C.A. (1994) Molecular characterization of microbial alcohol dehydrogenases. *Crit Rev Microbiol* **20**: 13–56. <https://doi.org/10.3109/10408419409113545>.
- Reisch, C.R., Moran, M.A., and Whitman, W.B. (2011) Bacterial catabolism of dimethylsulfoniopropionate (DMSP). *Front Microbiol* **2**: 1–12. <https://doi.org/10.3389/fmicb.2011.00172>.
- Riemer, D.D. (1998) *Marine and Terrestrial Sources of Reactive Volatile Organic Compounds and Their Impact on the Tropospheric Ozone Chemistry of the Earth*. USA: University of Miami. <https://ui.adsabs.harvard.edu/abs/1998PhDT.....49R>.
- Rowe, G.T., Sibuet, M., Deming, J., Tietjen, J., and Khrifounoff, A. (1990) Organic carbon turnover time in deep-sea benthos. *Prog Oceanogr* **24**: 141–160. [https://doi.org/10.1016/0079-6611\(90\)90026-X](https://doi.org/10.1016/0079-6611(90)90026-X).
- Rusch, D.B., Halpern, A.L., Sutton, G., Heidelberg, K.B., Williamson, S., Yooseph, S., et al. (2007) The sorcerer II global ocean sampling expedition: Northwest Atlantic through eastern tropical Pacific. *PLoS Biol* **5**: e77. <https://doi.org/10.1371/journal.pbio.0050077>.
- Sauer, K., Harms, U., and Thauer, R.K. (1997) Methanol: coenzyme M methyltransferase from methanosarcina barkei purification, properties and encoding genes of the corinoid protein MT1. *Eur J Biochem* **243**: 670–677. <https://doi.org/10.1111/j.1432-1033.1997.t01-1-00670.x>.
- Schink, B., and Zeikus, J.G. (1982) Microbial ecology of pectin decomposition in anoxic lake sediments. *Microbiology* **128**: 393–404. <https://doi.org/10.1099/00221287-128-2-393>.
- Schink, B., and Zeikus, J.G. (1980) Microbial methanol formation: a major end product of pectin metabolism. *Curr*

- Microbiol* **4**: 387–389. <https://doi.org/10.1007/BF02605383>.
- Schnell, S., Bak, F., and Pfennig, N. (1989) Anaerobic degradation of aniline and dihydroxybenzenes by newly isolated sulfate-reducing bacteria and description of *Desulfobacterium anilini*. *Arch Microbiol* **152**: 556–563. <https://doi.org/10.1007/BF00425486>.
- Schuppert, B., and Schink, B. (1990) Fermentation of methoxyacetate to glycolate and acetate by newly isolated strains of *Acetobacterium* sp. *Arch Microbiol* **153**: 200–204. <https://doi.org/10.1007/BF00247821>.
- Sela-Adler, M., Ronen, Z., Herut, B., Antler, G., Vigderovich, H., Eckert, W., and Sivan, O. (2017) Co-existence of methanogenesis and sulfate reduction with common substrates in sulfate-rich estuarine sediments. *Front Microbiol* **8**: 766. <https://doi.org/10.3389/FMICB.2017.00766>.
- Shi, L., Dong, H., Reguera, G., Beyenal, H., Lu, A., Liu, J., et al. (2016) Extracellular electron transfer mechanisms between microorganisms and minerals. *Nat Rev Microbiol* **14**: 651–662. <https://doi.org/10.1038/nrmicro.2016.93>.
- Sieburth, J.M., and Keller, M.D. (1989) Biological oceanography methylaminotrophic bacteria in xenic nanoalgal cultures: incidence, significance, and role of methylated algal osmoprotectants. *Biol Oceanogr* **6**: 383–395. <https://doi.org/10.1080/01965581.1988.10749541>.
- Singh, N., Kendall, M.M., Liu, Y., and Boone, D.R. (2005) Isolation and characterization of methylotrophic methanogens from anoxic marine sediments in Skan Bay, Alaska: description of *Methanococcoides alaskense* sp. nov., and emended description of *Methanosarcina baltica*. *Int J Syst Evol Microbiol* **55**: 2531–2538. <https://doi.org/10.1099/ijs.0.63886-0>.
- Sista Kameshwar, A.K., and Qin, W. (2018) Structural and functional properties of pectin and lignin-carbohydrate complexes de-esterases: a review. *Bioresour Bioprocess* **5**: 43. <https://doi.org/10.1186/s40643-018-0230-8>.
- Sousa, D.Z., Visser, M., Van Gelder, A.H., Boeren, S., Pieterse, M.M., Pinkse, M.W.H., et al. (2018) The deep-subsurface sulfate reducer *Desulfotomaculum kuznetsovii* employs two methanol-degrading pathways. *Nat Commun* **9**: 239. <https://doi.org/10.1038/s41467-017-02518-9>.
- Sowers, K.R., Baron, S.F., and Ferry, J.G. (1984) *Methanosarcina acetivorans* sp. nov., an acetotrophic methane-producing bacterium isolated from marine sediments. *Appl Environ Microbiol* **47**: 971–978. Retrieved from. <http://www.ncbi.nlm.nih.gov/pubmed/16346552>.
- Sowers, K.R., and Ferry, J.G. (1983) Isolation and characterization of a methylotrophic marine methanogen, *Methanococcoides methylutens* gen. nov, sp nov. *Appl Environ Microbiol* **45**: 684–690. Retrieved from. <http://www.ncbi.nlm.nih.gov/pubmed/16346215>.
- Stupperich, E. (1994) Corrinoid-dependent mechanism of acetogenesis from methanol. In *Acetogenesis*, Drake, H. L. (ed), pp. 180–194. New York: Chapman & Hall. <https://doi.org/10.1007/978-1-4615-1777-1>.
- Sun, J., Mausz, M.A., Chen, Y., and Giovannoni, S.J. (2019) Microbial trimethylamine metabolism in marine environments. *Environ Microbiol* **21**: 513–520. <https://doi.org/10.1111/1462-2920.14461>.
- Taubert, M., Grob, C., Crombie, A., Howat, A.M., Burns, O. J., Weber, M., et al. (2019) Communal metabolism by *Methylococcaceae* and *Methylophilaceae* is driving rapid aerobic methane oxidation in sediments of a shallow seep near Elba, Italy. *Environ Microbiol* **21**: 3780–3795. <https://doi.org/10.1111/1462-2920.14728>.
- Taubert, M., Grob, C., Howat, A.M., Burns, O.J., Pratscher, J., Jehmlich, N., et al. (2017) Methylamine as a nitrogen source for microorganisms from a coastal marine environment. *Environ Microbiol* **19**: 2246–2257. <https://doi.org/10.1111/1462-2920.13709>.
- Thauer, R.K., Jungermann, K., and Decker, K. (1977). Energy conservation in chemotrophic anaerobic bacteria. *Bacteriol Rev* **41**(1): 100–180. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC413997/>.
- Timmers, P.H.A., Welte, C.U., Koehorst, J.J., Plugge, C.M., Jetten, M.S.M., and Stams, A.J.M. (2017) Reverse methanogenesis and respiration in methanotrophic archaea. *Archaea* **2017**: 1654237. <https://doi.org/10.1155/2017/1654237>.
- Vaksmas, A., Guerrero-Cruz, S., van Alen, T.A., Cremers, G., Ettwig, K.F., Lücke, C., and Jetten, M.S.M. (2017) Enrichment of anaerobic nitrate-dependent methanotrophic ‘Candidatus Methanoperedens nitroreducens’ archaea from an Italian paddy field soil. *Appl Microbiol Biotechnol* **101**: 7075–7084. <https://doi.org/10.1007/s00253-017-8416-0>.
- van der Meijden, P., Heythuysen, H.J., Pouwels, A., Houwen, F., Van Der Drift, C., and Vogels, G.D. (1983) Methyltransferases involved in methanol conversion by *Methanosarcina barkeri*. *Arch Microbiol* **134**: 238–242. <https://doi.org/10.1007/BF00407765>.
- van der Meijden, P., van der Lest, C., van der Drift, C., and Vogels, G.D. (1984) Reductive activation of methanol: 5-Hydroxybenzimidazolylcobamide methyltransferase of *Methanosarcina barkeri*. *Biochem Biophys Res Commun* **118**: 760–766. [https://doi.org/10.1016/0006-291X\(84\)91460-8](https://doi.org/10.1016/0006-291X(84)91460-8).
- Vandieken, V., Knoblauch, C., and Jørgensen, B.B. (2006) *Desulfotomaculum arcticum* sp. nov., a novel spore-forming, moderately thermophilic, sulfate-reducing bacterium isolated from a permanently cold fjord sediment of Svalbard. *Int J Syst Evol Microbiol* **56**: 687–690. <https://doi.org/10.1099/ijs.0.64058-0>.
- Vandieken, V., Niemann, H., Engelen, B., and Cypionka, H. (2017) *Marinisporobacter balticus* gen. nov., sp. nov., *Desulfosporosinus nitroreducens* sp. nov. and *Desulfosporosinus fructosivorans* sp. nov., new spore-forming bacteria isolated from subsurface sediments of the Baltic Sea. *Int J Syst Evol Microbiol* **67**: 1887–1893. <https://doi.org/10.1099/ijs.0.001883>.
- Vanwonterghem, I., Evans, P.N., Parks, D.H., Jensen, P.D., Woodcroft, B.J., Hugenholtz, P., and Tyson, G.W. (2016) Methylotrophic methanogenesis discovered in the archaeal phylum Verstraetearchaeota. *Nat Microbiol* **1**: 1–9. <https://doi.org/10.1038/nmicrobiol.2016.170>.
- Velterop, J.S., Sellink, E., Meulenber, J.J.M., David, S., Bulder, I., and Postma, P.W. (1995) Synthesis of pyrroloquinoline quinone in vivo and in vitro and detection of an intermediate in the biosynthetic pathway. *J Bacteriol* **177**: 5088–5098. <https://doi.org/10.1128/jb.177.17.5088-5098.1995>.

- Villeneuve, C., Martineau, C., Mauffrey, F., and Villemur, R. (2013) *Methylophaga nitratreducenticrescens* sp. nov. and *Methylophaga frapperi* sp. nov., isolated from the biofilm of the methanol-fed denitrification system treating the seawater at the Montreal biodome. *Int J Syst Evol Microbiol* **63**: 2216–2222. <https://doi.org/10.1099/ijms.0.044545-0>.
- Visser, M., Pieterse, M.M., Pinkse, M.W.H., Nijse, B., Verhaert, P.D.E.M., de Vos, W.M., et al. (2016) Unravelling the one-carbon metabolism of the acetogen *Sporomusa* strain An4 by genome and proteome analysis. *Environ Microbiol* **18**: 2843–2855. <https://doi.org/10.1111/1462-2920.12973>.
- Von Klein, D., Arab, H., Völker, H., and Thomm, M. (2002) *Methanosarcina baltica*, sp. nov., a novel methanogen isolated from the Gotland Deep of the Baltic Sea. *Extremophiles* **6**: 103–110. <https://doi.org/10.1007/s007920100234>.
- Waite, D.W., Chuvochina, M., Pelikan, C., Parks, D.H., Yilmaz, P., Wagner, M., et al. (2020) Proposal to reclassify the proteobacterial classes Deltaproteobacteria and Oligoflexia, and the phylum Thermodesulfobacteria into four phyla reflecting major functional capabilities. *Int J Syst Evol Microbiol* **70**: 5972. <https://doi.org/10.1099/ijsem.0.004213>.
- Watanabe, M., Kojima, H., and Fukui, M. (2018) Review of Desulfotomaculum species and proposal of the genera Desulfallas gen. nov., Desulfofundulus gen. nov., Desulfofarcimen gen. nov. and Desulfohalotomaculum gen. nov. *Int J Syst Evol Microbiol* **68**: 2891–2899. <https://doi.org/10.1099/ijsem.0.002915>.
- Watkins, A.J., Roussel, E.G., Parkes, R.J., and Sass, H. (2014) Glycine betaine as a direct substrate for methanogens (*Methanococoides* spp.). *Appl Environ Microbiol* **80**: 289–293. <https://doi.org/10.1128/AEM.03076-13>.
- Wegener, G., Krukenberg, V., Ruff, S.E., Kellermann, M.Y., and Knittel, K. (2016) Metabolic capabilities of microorganisms involved in and associated with the anaerobic oxidation of methane. *Front Microbiol* **7**: 46. <https://doi.org/10.3389/fmicb.2016.00046>.
- Welander, P.V., and Metcalf, W.W. (2005) Loss of the mtr operon in *Methanosarcina* blocks growth on methanol, but not methanogenesis, and reveals an unknown methanogenic pathway. *Proc Natl Acad Sci U S A* **102**: 10664–10669. <https://doi.org/10.1073/pnas.0502623102>.
- Welte, C.U., Rasigraf, O., Vaksmaa, A., Versantvoort, W., Arshad, A., Op den Camp, H.J.M., et al. (2016) Nitrate- and nitrite-dependent anaerobic oxidation of methane. *Environ Microbiol Rep* **8**: 941–955. <https://doi.org/10.1111/1758-2229.12487>.
- Wilham, T., Zhilina, T.N., and Hummel, P. (1991) DNA-DNA hybridization of methylotrophic halophilic methanogenic bacteria and transfer of *Methanococcus halophilus* vp to the genus *Methanohalophilus* as *Methanohalophilus halophilus* comb. nov. *Int J Syst Bacteriol* **41**: 558–562. <https://doi.org/10.1099/00207713-41-4-558>.
- Williams, J., Holzinger, R., Gros, V., Xu, X., Atlas, E., and Wallace, D.W.R. (2004) Measurements of organic species in air and seawater from the tropical Atlantic. *Geophys Res Lett* **31**: L23S06. <https://doi.org/10.1029/2004GL020012>.
- Wu, M.L., Ettwig, K.F., Jetten, M.S.M., Strous, M., Keltjens, J.T., and van Niftrik, L. (2011) A new intra-aerobic metabolism in the nitrite-dependent anaerobic methane-oxidizing bacterium *Candidatus "Methylomirabilis oxyfera"*. *Biochem Soc Trans* **39**: 243–248. <https://doi.org/10.1042/BST0390243>.
- Yanagawa, K., Tani, A., Yamamoto, N., Hachikubo, A., Kano, A., Matsumoto, R., and Suzuki, Y. (2016) Biogeochemical cycle of methanol in anoxic deep-sea sediments. *Microbes Environ* **31**: 190–193. <https://doi.org/10.1264/jsm2.ME15204>.
- Yang, M., Nightingale, P.D., Beale, R., Liss, P.S., Blomquist, B., and Fairall, C. (2013) Atmospheric deposition of methanol over the Atlantic Ocean. *Proc Natl Acad Sci U S A* **110**: 20034. Retrieved from. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3864313/>.
- Zhang, W., Zhang, T., Wu, S., Wu, M., Xin, F., Dong, W., et al. (2017) Guidance for engineering of synthetic methylotrophy based on methanol metabolism in methylotrophy. *RSC Adv* **7**: 4083–4091. <https://doi.org/10.1039/c6ra27038g>.
- Zhuang, G., Xu, L., Liang, Q., Fan, X., Xia, Z., Joye, S.B., and Wang, F. (2019b) Biogeochemistry, microbial activity, and diversity in surface and subsurface deep-sea sediments of South China Sea. *Limnol Oceanogr* **64**: 2252. <https://doi.org/10.1002/lno.11182>.
- Zhuang, G.C., Heuer, V.B., Lazar, C.S., Goldhammer, T., Wendt, J., Samarkin, V.A., et al. (2018a) Relative importance of methylotrophic methanogenesis in sediments of the Western Mediterranean Sea. *Geochim Cosmochim Acta* **224**: 171–186. <https://doi.org/10.1016/j.gca.2017.12.024>.
- Zhuang, G.-C., Lin, Y.-S., Elvert, M., Heuer, V.B., and Hinrichs, K.-U. (2014) Gas chromatographic analysis of methanol and ethanol in marine sediment pore waters: validation and implementation of three pretreatment techniques. *Mar Chem* **160**: 82–90. <https://doi.org/10.1016/J.MARCHEM.2014.01.011>.
- Zhuang, G.C., Montgomery, A., Samarkin, V.A., Song, M., Liu, J., Schubotz, F., et al. (2019a) Generation and utilization of volatile fatty acids and alcohols in hydrothermally altered sediments in the Guaymas Basin, Gulf of California. *Geophys Res Lett* **46**: 2637–2646. <https://doi.org/10.1029/2018GL081284>.
- Zhuang, G.-C., Peña-Montenegro, T.D., Montgomery, A., Hunter, K.S., and Joye, S.B. (2018b) Microbial metabolism of methanol and methylamine in the Gulf of Mexico: insight into marine carbon and nitrogen cycling. *Environ Microbiol* **20**: 4543. <https://doi.org/10.1111/1462-2920.14406>.

### 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.