Environmental Applications of Methanotrophs

Adrian Ho, Miye Kwon, Marcus A. Horn, and Sukhwan Yoon

Contents

Abstract Methanotrophs are microorganisms that are able to utilize methane as the electron donor and carbon source. For long, methanotrophs have been widely studied for their application in environmental biotechnology, due mainly to the exclusive ownership of the unique enzymes that mediate oxidation of methane to methanol, namely the particulate methane monooxygenases (pMMO) and soluble methane monooxygenases (sMMO). Utilizing these methane monooxygenases, methanotrophs are capable of co-oxidizing a broad range of organic pollutants including chlorinated ethenes. Thus, methanotrophs have long been studied and utilized as biocatalysts for in situ bioremediation of soil and aquatic environments contaminated with these xenobiotic compounds. Due to the growing concerns in anthropogenically induced climate change and global warming, methanotrophs have increasingly gained attention also for greenhouse gas mitigation purposes. Active methane removal using methanotrophic biofilters of diverse configurations have proven to be effective for treatments of gases with relatively high methane concentrations, e.g., landfill gases and animal husbandry tank exhausts. Furthermore, improving the atmospheric methane sink capability of agricultural soils has been one of the foremost foci of climate-smart soil research. This

A. Ho · M. A. Horn
Institute for Microbiology, Leibniz Universität Hannover, Hannover, Germany
e-mail: adrian.ho@fmb.uni-hannover.de; horn@ifmb.uni-hannover.de

M. Kwon · S. Yoon (✉)
Department of Civil and Environmental Engineering, KAIST, Daejeon, South Korea
e-mail: miyekwon@kaist.ac.kr; syoon80@kaist.ac.kr

E. Y. Lee (ed.), *Methanotrophs*, Microbiology Monographs 32,
https://doi.org/10.1007/978-3-030-23261-0_8

chapter provides an extensive overview of scientific and engineering breakthroughs geared towards practical applications of methanotroph biotechnology in managing impending environmental problems.

1 Introduction

Methanotrophs are groups of organisms that are capable of utilizing methane as the energy and carbon source (Hanson and Hanson 1996; Semrau et al. 2010). Traditionally, only two subgroups belonging to the *Proteobacteria* phylum (aerobic alphaproteobacterial and gammaproteobacterial methanotrophs) had been recognized as methanotrophs; however, the term 'methanotroph' is now used in a broader context, encompassing the aerobic verrucomicrobial methanotrophs, anaerobic archaeal methanotrophs, and denitrifying methanotrophs of the NC10 phylum, besides the conventional proteobacterial methanotrophs (Raghoebarsing et al. 2006; Knittel and Boetius 2009; Op den Camp et al. 2009). These organisms, except for the archaeal anaerobic methanotrophs, possess and utilize particulate (pMMO) and/or soluble methane monooxygenases (sMMO), which are the only identified enzymes capable of catalyzing CH_4 oxidation to CH_3OH (Semrau et al. 2010). Most, if not all, previous studies on environmental applications of methanotrophs have focused on aerobic proteobacterial methanotrophs possessing these MMOs. Therefore, the literature review in this chapter will be limited mostly to these conventional aerobic methanotrophs, despite the recently invigorated scientific interests in verrucomicrobial methanotrophs and anaerobic methanotrophy.

The methane monooxygenases are key to environmental applications of methanotrophs (Jiang et al. 2010; Wendlandt et al. 2010). Both pMMO and sMMO have the identical physiological function of catalyzing CH_4 turnover to CH_3OH in methanotrophs; however, these two groups of MMOs have distinct evolutionary history and do not share any structural similarity (Elango et al. 1997; Leahy et al. 2003; Lieberman and Rosenzweig 2005; Khadka et al. 2018). While pMMO are membrane-integrated cuproenzymes that share the evolutionary lineage with ammonia monooxygenases (AMO), sMMO are structurally and evolutionarily affiliated to soluble di-iron monooxygenases, which include diverse alkene/aromatic monooxygenases (Leahy et al. 2003; Khadka et al. 2018). Ecological studies have suggested that pMMO is the prevalent form of methane monooxygenases in most environments inhabited by proteobacterial methanotrophs. The *pmoA* gene, encoding for the pMMO, has been recovered in a much larger quantity than the *mmoX* gene which encodes for the sMMO, in most soil and aquatic environments examined to date (Rahman et al. 2011). Until recently, no RNA-targeted analysis had been successful in detecting *mmoX* transcript in environmental samples, suggesting that the *mmoX* is rarely transcribed by methanotrophs in natural environmental settings (Chen et al. 2007; Liebner and Svenning 2013; Kumaresan et al. 2018). All isolated proteobacterial methanotrophs, except for the strains belonging to the *Methylocella*

and *Methyloferula* genera, possess pMMO. Contrastingly, the genes encoding sMMO are only found in selected subgroups of methanotrophs across diverse phylogenetic groups within the *Proteobacteria* (Semrau et al. 2010; Kenney et al. 2016). These different lines of evidence point towards pMMO being more relevant to overall methanotrophic activity in natural environments, although in vitro incubation in Cu-depleted medium elevates sMMO expression and activity in methanotrophs possessing both MMOs, i.e. *Methylosinus trichosporium* strain OB3b and *Methylococcus* strain Bath (Murrell et al. 2000; Kenney et al. 2016).

Attempts to artificially generate methane-oxidizing bacteria using synthetic biology approaches have been unsuccessful, and the methanotrophs of the phyla *Proteobacteria*, *Verrucomicrobia*, and NC10 remain as the only groups of organisms capable of wielding either MMO. Ex vivo activities of isolated MMO enzymes, both sMMO and pMMO, have been examined, but without success in reproducing in vivo activity levels or maintaining their reactivity over extended period of time (Fox et al. 1989; Zahn and DiSpirito 1996). Thus, to utilize MMOs for environmental applications, whole-cell activities of the sMMO- or pMMO-expressing methanotrophs appears as the most feasible option. Such application of methanotrophs capitalize on largely two catalytic capabilities of the MMOs: CH_4 oxidation to CH_3OH, and involuntary cometabolic mono-oxidation of various mono- and multi-carbon organics. During the decades spanning 1980s through 2000s, when the public interest in environmental bioremediation was at its apex, cometabolic degradation of organic contaminants was the foremost research focus of methanotroph biotechnology (Semrau 2011). More recently, however, with the growing concern in global warming and climate change, the focus has shifted towards biological mitigation of CH_4 emissions from its major sources, e.g., landfills, animal feeding operations, and rice paddies. Besides, recent research aims to develop strategies to augment the methane sink function of the indigenous high-affinity methanotrophs in agricultural soils. In this chapter, the history of methanotroph biotechnology is summarized and the future research directions geared towards practical applications of methanotrophs are posited.

2 Cometabolic Organic Pollutant Degradation

Methanotrophs are capable of degrading various organic compounds utilizing methane monooxygenases. Particularly, their capability to oxidize various halogenated organic compounds has been widely studied. The most commonly practiced approach for bioremediation of chlorinated organic compounds has been the reductive dechlorination approach, whereby dechlorination serve as the electron acceptor reaction for selected groups of anaerobic microorganisms, e.g., *Dehalobacter* spp. and *Dehalococcoides* spp. (Holliger et al. 1998; Löffler et al. 2013). The mechanism of methanotrophic cometabolic degradation is entirely distinct from that of reductive dehalogenation, in that dehalogenation occurs via an oxidative pathway with O_2 as the electron acceptor (Keck et al. 1989; Alvarez-Cohen and Speitel 2001). Unlike in

reductive dechlorination, in which halogenated organic compounds are utilized as growth substrates, these organic contaminants cannot serve as growth substrates for methanotrophs and can be co-oxidized only in the presence of CH_4. Thus, supply of CH_4 (the electron donor and carbon source) and O_2 (electron acceptor) need to be ensured to maintain activity of methanotrophs as they cometabolically oxidize halogenated organic compounds.

Methanotrophic co-oxidation of halogenated hydrocarbons is catalyzed by MMOs (Semrau et al. 2010). In general, the copper-free sMMOs have been observed with higher reactivity towards non-CH_4 substrates than the copper-dependent pMMOs (Lee et al. 2006; Yoon and Semrau 2008). Thus, initial research efforts for halogenated hydrocarbon degradation were focused on utilization of sMMO-expressing methanotrophs (Oldenhuis et al. 1991; van Hylckama Vlieg et al. 1996). Although the high metabolic rate of sMMO towards chlorinated hydrocarbons is certainly attractive, the sMMO-utilizing approach has limitations in its practical applications. The expression of sMMO is tightly regulated by copper and the near-complete absence of copper necessitated for sMMO expression is rare in terrestrial environments (Murrell et al. 2000). In fact, *mmoX* transcripts are rarely detected in molecular analyses targeting RNA in CH_4-oxidizing soils (Chen et al. 2007, 2008; Lee et al. 2009). Thus, the effectiveness of sMMO-catalyzed halogenated hydrocarbon oxidation is questionable in in situ bioremediation. As cometabolic substrates are often competitive inhibitors to methane oxidation and cometabolic oxidation often generates toxic intermediates, the high affinity and reactivity of sMMO towards chlorinated hydrocarbon compounds may act against establishment of methanotroph population when the contaminants are present at high concentrations (Alvarez-Cohen and McCarty 1991; Lee et al. 2006). Therefore, several researches have investigated pMMO-mediated halogenated hydrocarbon as an alternative, despite of the low reactivity of pMMO towards non-CH_4 compounds (Anderson and McCarty 1997; Lontoh and Semrau 1998; Han et al. 1999).

The xenobiotic halogenated hydrocarbons targeted by methanotrophic cometabolic degradation and the kinetic parameters experimentally determined with laboratory strains of methanotrophs expressing sMMO or pMMO are summarized in Table 1. Majority of these biodegradation experiments were performed with *M. trichosporium* OB3b (*Alphaproteobacteria*) and *M. capsulatus* Bath (*Gammaproteobacteria*) at the sMMO-expressing condition, i.e., in absence of copper. Degradation of trichloroethylene (TCE), *cis*- and *trans*-dichloroethylene (*c*- and *t*-DCE), and vinyl chloride (VC), among many other one- or two-carbon halogenated compounds have also been confirmed with pMMO-expressing methanotrophs, including those lacking *mmoX* gene (Han et al. 1999).

Biodegradation of the chlorinated ethenes using the methanotrophic cometabolism attracted particular interests during the decades spanning the 1980s to 2000s. Although methanotrophic cometabolic degradation had a major drawback in its field application, in that it demands continuous supply of CH_4 and O_2 to the target contaminated sites, cometabolic degradation has certain advantages over reductive dechlorination in removal of chlorinated ethenes. The most toxic form of the chlorinated ethenes is VC. Even though *Dehalococcoides* spp. capable of

Table 1 Apparent whole-cell Michaelis-Menten rate constants determined for cometabolic degradation of halogenated hydrocarbons by methanotrophs expressing either particulate or soluble methane monooxygenases

Compound[a]	Methanotroph[b]	MMO[c]	V_{max} [nmol min^{-1}·(mg protein)$^{-1}$][d]	K_m (μM)	References
TCE	BG8	pMMO	4.3	59	Han et al. (1999)
	OB3b	pMMO	214	15	Lontoh et al. (1999)
	OB3b	sMMO	580[e]	145	Oldenhuis et al. (1991)
	Mixed	pMMO	1.3	44	Forrester et al. (2005)
VC	BG8	pMMO	7	30	Han et al. (1999)
	OB3b	pMMO	42	26	Lee et al. (2006)
	OB3b	sMMO	2100	160	Lee et al. (2006)
	Mixed	pMMO	9.9	47	Forrester et al. (2005)
c-DCE	BG8	pMMO	0.12	0.8	Han et al. (1999)
	Mixed	pMMO	0.52	3.1	Forrester et al. (2005)
	OB3b	sMMO	364[e]	30	Oldenhuis et al. (1991)
t-DCE	BG8	pMMO	43	60	Han et al. (1999)
	OB3b	sMMO	662[e]	148	Oldenhuis et al. (1991)
	OB3b	pMMO	61	42	Lee et al. (2006)
1,1-DCE	BG8	pMMO	0.23	2.5	Han et al. (1999)
	OB3b	sMMO	12[e]	5	Oldenhuis et al. (1991)
DCM	BG8	pMMO	33	73	Han et al. (1999)
	OB3b	pMMO	235	32	Lontoh et al. (1999)
	OB3b	sMMO	66[e]	4	Oldenhuis et al. (1991)

(continued)

Table 1 (continued)

Compound[a]	Methanotroph[b]	MMO[c]	V_{max} [nmol min^{-1}·(mg protein)$^{-1}$][d]	K_m (μM)	References
CF	OB3b	sMMO	1096[e]	34	Oldenhuis et al. (1991)
DBM	BG8	pMMO	45	171	Han et al. (1999)
1,1,1-TCA	OB3b	sMMO	48	214	Oldenhuis et al. (1991)

[a]TCE, trichloroethylene; VC, vinyl chloride; *c*-DCE, *cis*-dichloroethylene; *t*-DCE, *trans*-dichloroethylene; 1,1-DCE, 1,1-dichloroethylene; DCM, dichloromethane; BF, bromoform; CF, chloroform; DBM, dibromomethane; 1,1,1-TCA, 1,1,1-trichloroethane

[b]BG8, *Methylomicrobium album* strain BG8; OB3b, *Methylosinus trichosporium* strain OB3b; Mixed, Uncharacterized mixed methanotrophic culture

[c]pMMO, particulate methane monooxygenase; sMMO, soluble methane monooxygenase

[d]Methanotrophs were cultured at 30 °C with addition of 20 mM formate

[e]Converted from the reported units of mL min^{-1} (mg cells)$^{-1}$, assuming that 50% of the total cell dry weight is protein

reducing VC (C_2H_3Cl) to innocuous ethene (C_2H_4) have been discovered and microbial consortia capable of complete reduction of chlorinated ethenes have been fully commercialized, VC remains to be the most recalcitrant intermediate in the reductive dechlorination pathway (He et al. 2003a, b; Ritalahti et al. 2005; Popat and Deshusses 2009). Thus, VC accumulation is unavoidable in bioremediation practices employing reductive dechlorination. In methanotrophic cometabolism, regardless of whether sMMO or pMMO is involved, oxidization of TCE and *c*- and *t*-DCE does not leave stable toxic intermediates and VC is the most rapidly degraded compound of the chlorinated ethenes (van Hylckama Vlieg and Janssen 2001; Lee et al. 2006).

Methanotrophs are also less vulnerable to adverse changes to environmental conditions than VC-respiring *Dehalococcoides* spp., which are easily inactivated by O_2 exposure or modest changes to pH or salinity (Amos et al. 2008; Islam et al. 2016; Matturro et al. 2016; Yang et al. 2017a; Ho et al. 2018). Methanotrophic degradation of chlorinated ethenes have been examined only at the neutral-pH conditions; however, diverse groups of acidophilic and halophilic methanotrophs harboring pMMO and/or sMMO have been isolated, suggesting that acidophilic or halophilic biological degradation of chlorinated ethenes, including VC, may be feasible (Kip et al. 2011; Semrau 2011). No VC-to-ethene reduction activity has been observed in acidic VC-to-ethene reduction or saline environments to date (Kittelmann and Friedrich 2008; Yang et al. 2017b). Reductive dehalogenation of chlorinated ethenes at pH 5.5 had resulted in permanent stoichiometric accumulation of VC in *D. mccartyi* cultures, suggesting complete inactivation of VC reductase and potential VC accumulation from PCE or TCE in contaminated acidic soil (Yang et al. 2017a). Biostimulation or, if necessary, bioaugmentation of acidophilic or halophilic methanotrophs may be a plausible alternative for removal of residual VC from such environments recalcitrant to the reductive dechlorination approach.

Several different field application methods were tested as the means to stimulate methanotrophic cometabolism in situ for removal of chlorinated ethenes. In the pilot scale experiment performed at Moffett Naval Air Station using biostimulation approach, the methanotrophic activity in the artificially contaminated aquifer was stimulated in situ by alternating pulse injections of air and CH_4 at the injection wells (Fig. 1a). The concentrations of TCE, *t*-DCE, and VC at the extraction wells were significantly lower with the air and CH_4 injections than without, indicating the occurrence of cometabolic biodegradation at the site. The bioremediation of the PCE/TCE-contaminated Savannah River Site employed horizontal injection wells installed below the water table for direct injection of CH_4/air mixed gas and horizontal vacuum extraction wells positioned in the vadose zone to capture stripped chlorinated ethenes for incineration (Fig. 1b) (Brockman et al. 1995; Hazen et al. 1993). Addition of CH_4/air mixed gas and gaseous nitrogen (nitrous oxide) and phosphorous (triethyl phosphate) sources resulted in active TCE/PCE removal in situ. Similar pilot scale studies using similar biostimulation approaches proved to be effective in removing chlorinated ethenes from contaminated soils and groundwater (Eguchi et al. 2001; Takeuchi et al. 2005).

In an attempt to avoid the drawbacks of such biostimulation strategy (e.g., inconsistent contacts between contaminant and stimulated bacterial population, spatially uneven biostimulation, and competitive inhibition of MMO's methane oxidation activity by chlorinated ethenes), an unconventional bioaugmentation approach was also tested (Taylor et al. 1993; Duba et al. 1996). Dense pre-incubated cultures of *M. trichosporium* OB3b were applied to porous media to establish in situ microbial filters and TCE-contaminated plumes were passed through these microbial filters. In both laboratory-scale experiments and the pilot-scale study at a TCE-contaminated site at the Chico Municipal Airport, TCE removal activities of the biofilters were sustained for >20 days, albeit with the removal efficiency gradually decreasing over time.

Whether sMMO- or pMMO-expressing methanotrophs are involved in the observed in situ chlorinated ethene degradation still remains controversial, as no comparative quantification of *pmoA* and *mmoX* transcripts or expressed proteins in situ has been reported to date. The presence of *mmoX* transcripts in the Savannah test site was confirmed with gene probes; however, due to the lack of information with regards to the quantitative comparison between *pmoA* and *mmoX* transcripts, the relative contribution of sMMO activity to TCE degradation remained unknown (Hazen et al. 2009).

3 Potential Environmental Applications of Methanobactin

The majority of methanotrophs in the environment utilize pMMO to carry out the first step of CH_4 oxidation and thus require sufficient supply of Cu^{2+} ions. Some alphaproteobacterial methanotrophs, previously termed type II methanotrophs, are capable of synthesizing and utilizing a Cu^{2+}-chelator termed methanobactin (Mbn)

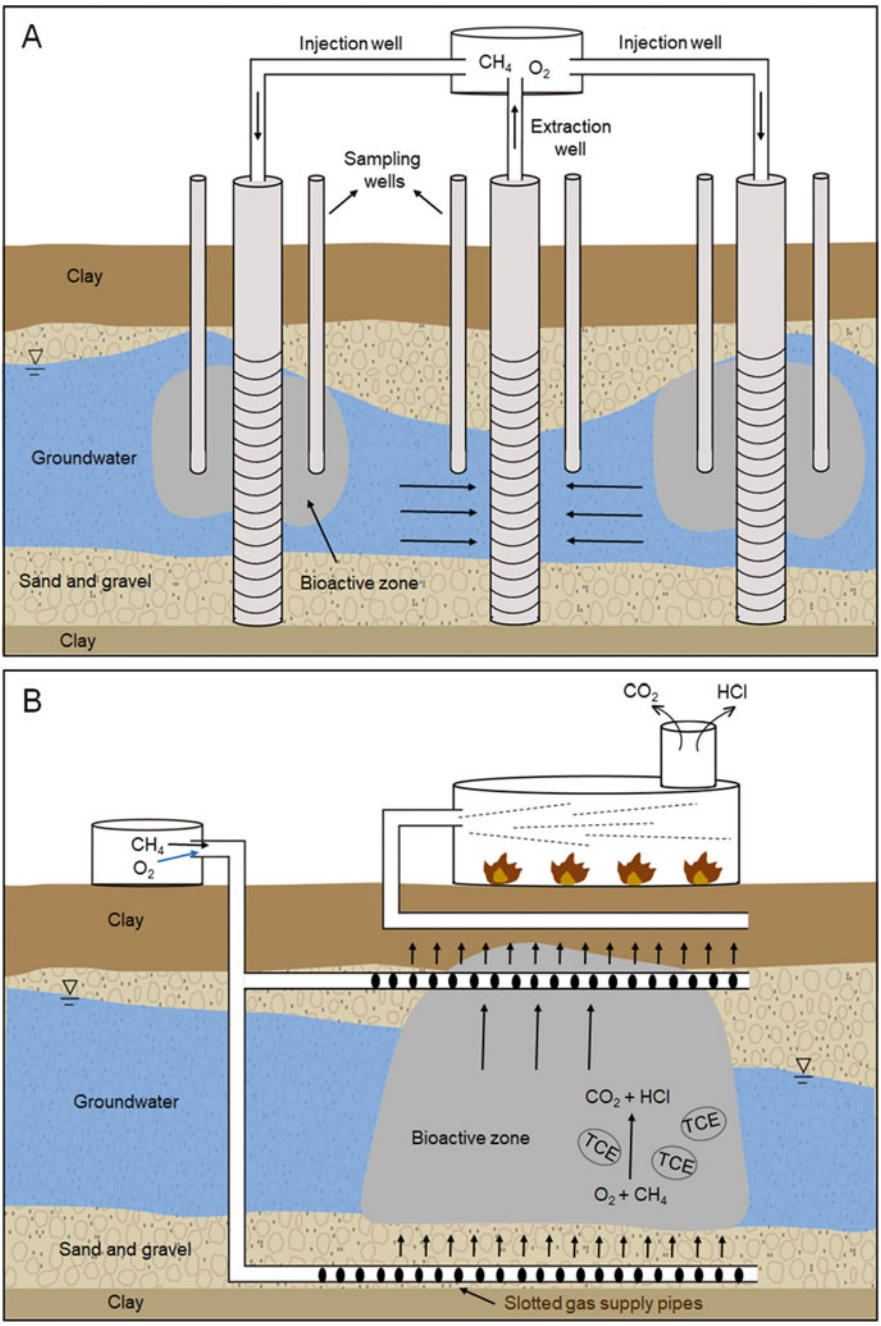

Fig. 1 Schematic depictions of the pilot-scale field tests of bioremediation of TCE-contaminated groundwater and soil via in situ CH_4 biostimulation of indigenous methanotrophs. (**a**) The field test at Moffet Naval Air Base, where pulse injections of air- and CH_4-saturated groundwater at the injection wells supported TCE removal from contaminated groundwater. (**b**) The field test at Savannah River Site, where mixed gas of CH_4 and air was supplied into contaminated aquifer

(Kim et al. 2004; DiSpirito et al. 2016). Recent studies of Mbn and Mbn-synthesizing methanotrophs have suggested several potential environmental applications of this novel copper-binding compound. Although Mbn binds Cu ions with high specificity, Mbn also forms complexes with other trace metals, most notably Hg^{2+} (Baral et al. 2014). Abstraction of Hg^{2+} by Mbn mitigated the mercury toxicity to methanotrophs of diverse phylogenetic groups, including those not able to synthesize Mbn (Vorobev et al. 2013); however, whether Mbn-bound Hg is less bioavailable and toxic to other micro- or macro-organisms still awaits to be examined. More recently, the Mbn-producing *M. trichosporium* strain OB3b was examined for the capability to uptake and demethylate methyl-mercury (CH_3Hg^+) (Lu et al. 2017). Methanobactin was found to have a crucial role in the demethylation reaction, as the *mbnA*$^-$ deletion mutant defective in the capability to Mbn lacked the capability. These experimental evidences suggest the possibility of utilizing Mbn or Mbn-producing methanotrophs for detoxifying mercury in heavy-metal contaminated sites.

Quite a few of the most consequential reactions in the biological N-cycling are catalyzed by cuproenzymes, i.e., the enzymes that require Cu for their activities. Ammonia monooxygenases (AMO), NirK-type NO_2^- reductases, and NosZ-type N_2O reductases are all crucial enzymes in the N-cycling that require Cu for their activities (Hulse et al. 1989; Brown et al. 2000; Khadka et al. 2018). Whether Mbn-bound Cu^{2+} can be taken up by non-methanotrophic organisms has not yet been closely examined. If the ability to uptake and utilize Mbn-bound Cu is not a widespread trait among the organisms wielding aforementioned cuproenzymes, Cu sequestration by Mbn may significantly alter the nitrogen flux in the environment. Indeed, in a recent experiment investigating the effect of Mbn on denitrification, the activities of NosZ and NirK were evidently hindered by the presence of Mbn (Chang et al. 2018). It is certainly plausible that AMO activities may also be affected by the presence of Mbn or Mbn-synthesizing methanotrophs. Such alteration of the nitrogen cycling reactions may have utilities in environmental engineering and agricultural practices. Methanobactin-induced inhibition of N_2O reduction can be utilized for recovery of N_2O from wastewater treatment plants with biological nitrogen removal (BNR) systems. Theoretically, denitrification can be stopped at N_2O by adding Mbn-enriched additives to anoxic tanks of activated sludge reactors. N_2O has been studied as the replacement for hydrazine as rocket fuels and has newfound utility in manufacturing of organic light emitting diode (OLED) displays (Yamazaki et al. 2014; Zakirov et al. 2001). Recovery of such valuable resource from otherwise wasted nitrogenous contaminants is crucial in developing more sustainable sewage treatment systems. The impacts of Mbn on ammonia oxidizing bacteria and ammonia oxidizing archaea have not yet been examined, but two possible effects can be

Fig. 1 (continued) through horizontal injection wells. The rising gas containing stripped TCE was extracted with another set of overlying horizontal injection wells and incinerated before release. The figures (**a**) and (**b**) were adapted from the graphical presentations in Semprini and McCarty (1991) and Hazen et al. (1993), respectively

expected. Methanobactin may suppress NH_3 oxidation by sequestering Cu ions away from ammonia monooxygenase-utilizing ammonia oxidizing bacteria and archaea, as was observed with NosZ-utilizing denitrifiers. Another possible outcome is protection of these sensitive organisms from copper and heavy metal toxicity (Park and Ely 2008). Either way, Mbn may prove useful, as a nitrification inhibitor for enhancement of nitrogen fertilizer efficiency or NH_3 oxidation facilitator in wastewater treatment plants debilitated by heavy metal influx.

4 Methane Emission Mitigation

The main growth substrate of methanotrophs, CH_4, is a potent greenhouse gas (GHG) with ~25 times higher global warming potential than CO_2 over 100-year period and the International Panel on Climate Change (IPCC) estimated the contribution of CH_4 to the net global GHG to be approximately 16% (Ciais et al. 2013). The largest anthropogenic CH_4 sources include landfills, livestock farming, rice farming, and mining industry (Ciais et al. 2013). Utilization of methanotrophic metabolism has long been considered as a viable strategy for mitigation of CH_4 emissions from these sources (summarized in Table 2).

Table 2 Lab- and field-scale experimentations of various CH_4 emission mitigation strategies utilizing CH_4-oxidizing capability of methanotrophs

Targeted source	Strategy	Scale	Materials	Methane removal rate (g h^{-1} m^{-3})	References
Landfill	Biofilter (active)	Pilot-scale	Loamy sand, clay, gravel	80	Gebert and Gröngröft (2006)
Landfill	Biofilter (passive)	Lab-scale	Compost	63	Streese and Stegmann (2003)
Landfill	Biofilter (passive)	Lab-scale	Inorganic material	13.3–29.2	Nikiema et al. (2005)
Manure storage	Biofilter (passive)	Pilot-scale	Perlite, garden compost	5.5–22	Melse and van der Werf (2005)
Enteric fermentation	Bio-integrated building material	Lab-scale	Aerated concrete	<0.01	Ganendra et al. (2014)
Enteric fermentation	Biofilter (passive)	Pilot-scale	Aerated concrete	2.8	Ganendra et al. (2015)
Dairy farm	Biofilter (passive)	Pilot-scale	Landfill cover soil, perlite	16	Pratt et al. (2012)
Anaerobic digester	Biofilter (passive)	Lab-scale	Activated carbon	348	Wu et al. (2017)

Landfill gases (LFG) with high CH_4 contents (e.g., >30%) are often collected and utilized for energy production and those with lower CH_4 contents are mostly flared before being released to the atmosphere. Neither is applicable, however, for the landfills gases with CH_4 contents below the flammable threshold (the lower flammability limit for CH_4 is 5.0% in air at 25 °C). Both passive biofiltration and forced-ventilation biofiltration have been devised and tested for CH_4 removal from the low-CH_4 LFG. The best example of the former is the pilot-scale biofilter tested for treatment of passively emitted gas from a low-organic land disposal site in Hamburg, Germany (Gebert et al. 2003). The biofilter was built into the landfill cover soils and filled with layers of gravels, sand and clay pellets and operated without artificial watering or nutrient supply (Fig. 2a). In spite of the reduced performance during the wintry months, the biofilter removed 62% of CH_4 emitted during the 1-year span of the test period (Gebert and Gröngröft 2006). The fatty acid profile-based analysis of the microbial composition indicated that the CH_4-oxidizing population in the biofilter was enriched with alphaproteobacterial methanotrophs of the *Methylosinus* and *Methylocystis* genera (Gebert et al. 2004).

Several researchers have examined CH_4-removal capacities of lab-scale and/or pilot-scale biofiltration experiments using forced ventilation with higher volumetric processing rates (Streese and Stegmann 2003; Nikiema et al. 2005). The lab-scale CH_4 biofilter developed by Nikiema et al. (2005) was constructed to the height and the internal diameter of 135 cm (subdivided into three sections of 45 cm) and 15 cm, respectively, and was operated as a biotrickling biofilter wetted with nutrient solution fed at the rate of 1.5 L day^{-1} (Fig. 2b). For treating 4.2 L min^{-1} gas influx containing 7000–7500 ppmv CH_4, CH_4-to-CO_2 conversion rates of up to 41% and elimination capacities up to 29 g m^{-3} h^{-1} were achieved with the biofilter packed with inorganic filter beds. The biofiltration system developed by Streese and Stegmann (2003) used a mixture of squeezed wood fibers, peat, and compost as the filter material and instead of wetting the filter beds with trickled nutrient medium, LFG was humidified in a separate antechamber with scrubbers before being distributed into the parallel biofilters. The pilot-scale biofiltration system with the total filter volume of 4 m^3 treated a mixed stream carrying 0.2–2.5% CH_4 with 25 m^3 h^{-1} total flow rate at an elimination capacity of 20–40 g m^{-3} h^{-1}.

Methane emissions from livestock farming have become increasingly problematic, due to the recent growth in global demands for meat and dairy products (Godfray et al. 2010). Enteric fermentation in cattle rumens is estimated to be responsible for approximately 25% of the current total anthropogenic CH_4 emissions, and additional CH_4 emission results from manure storage and processing (Ciais et al. 2013; Hou et al. 2015). Utilization of methanotrophs has been, for long, considered as a viable strategy for reducing CH_4 emissions from livestock farming (Melse and van der Werf 2005; Yoon et al. 2009; Ganendra et al. 2015). Melse and van der Werf (2005) examined the feasibility of CH_4 removal from off-gas from a liquid manure storage tank with a pilot-scale biofilter packed with a mixture of perlite and compost. Presumably due to the low temperature (average of 12.0 °C during the period of study) and the low CH_4 concentrations (<5 g m^{-3}), the observed elimination capacities were lower than the biofilters developed for LFG treatments. Nevertheless,

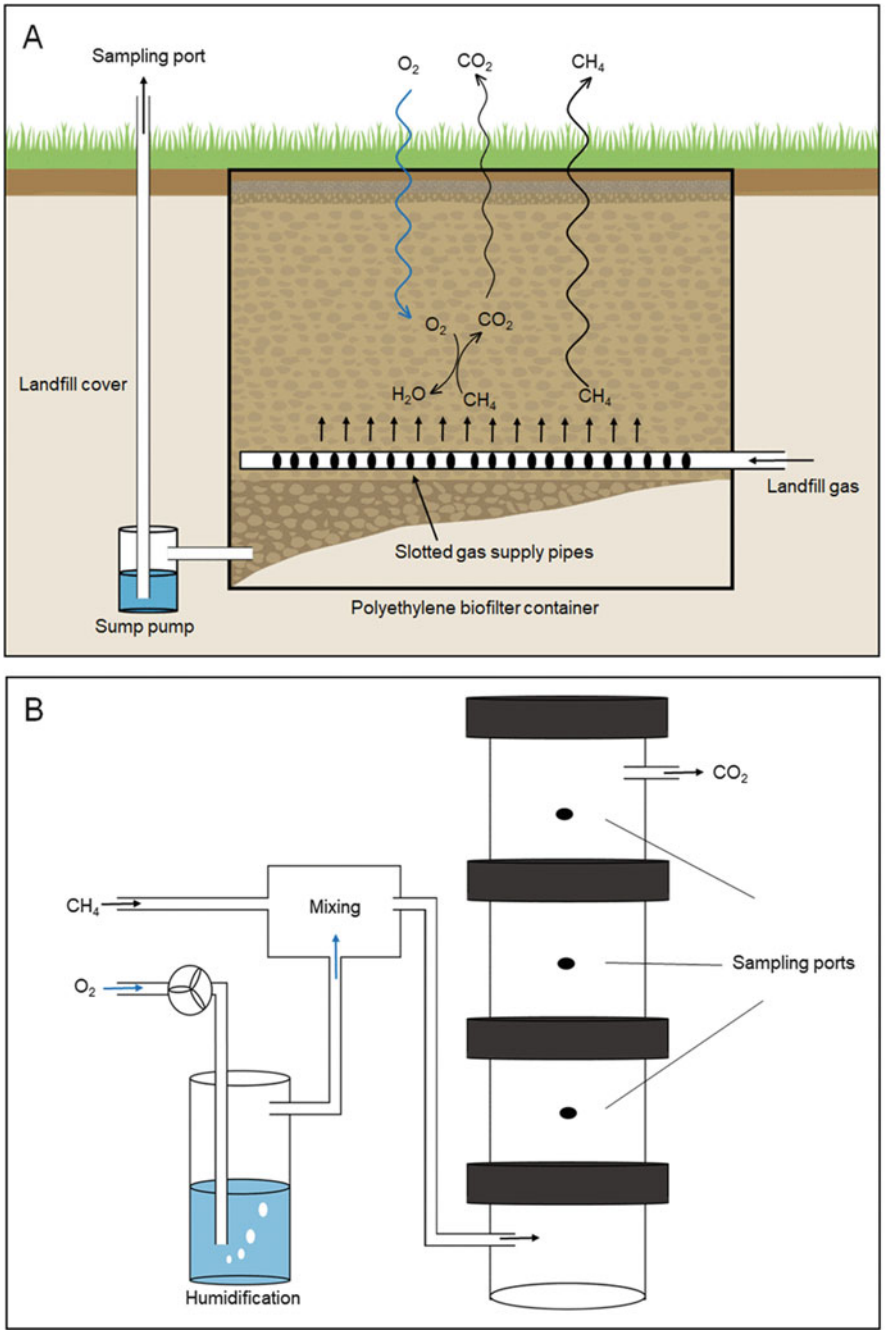

Fig. 2 Schematics of (**a**) a passive biofilter and (**b**) a forced-ventilation biofilter designed and experimented for removal of CH_4 from landfill gas. The figures (**a**) and (**b**) were adapted from Gebert et al. (2003) and Nikiema et al. (2005), respectively

substantial removal of CH_4 was possible using this approach at an estimated cost of $26 per ton CO_2eq. Incorporation of immobilized methanotrophs into the porous building materials has also been examined as an alternative strategy (Ganendra et al. 2014). Of the porous materials tested as the potential support for methanotrophic biofilms, aerated concrete was determined as the most suitable material, as the highest rates of CH_4 oxidation was observed at both high (*ca.* 20% v/v) and low (*ca.* 50 ppmv) concentrations of CH_4 with methanotrophs immobilized in this medium (Ganendra et al. 2014). A biofilter using this same material as the filter bed was also tested in ruminant respiration chambers; however, the device failed to achieve sufficiently high removal efficiency (Ganendra et al. 2015).

Similar methanotroph-utilizing approaches have been devised and tested for removal of CH_4 from other environmental hotspots, which include coal mines and oil sands tailings ponds (Limbri et al. 2014; La et al. 2018). Methane biofiltration may also provide a solution for the CH_4 leakage problem in shale gas mining, although no reliable literature has been published regarding such possibility (Alvarez et al. 2012). As such, microbial conversion of CH_4 to CO_2 remains to be one of the most realistic approaches for GHG emission reduction in the foreseeable future. Provided that microbial CH_4 emission reduction can be coupled to resource recovery, further cuts in the net costs will be possible. Such possibilities abound; methanotrophs (*M. capsulatus* Bath) have long been used for production of commercial single-cell protein and methanobactin has commercial potential as a cost-efficient cure for Wilson's disease (Lichtmannegger et al. 2016). Single cell protein and/or methanobactin can be collected from methanotrophic biomass grown in the CH_4 removal devices and processed for commercial production, relieving, at least in part, financial burden for the prospective operators.

The major dilemma in utilizing methanotrophic activity for reducing CH_4 emissions is the oft low concentration of emitted CH_4 (Scheutz et al. 2009; Yoon et al. 2009; Ganendra et al. 2015). The reported observed half-saturation constants ($K_{m,app}$) for CH_4 oxidation by conventional laboratory strains of methanotrophs, range from 0.11 to 23 μM (equivalent to 77–16,100 ppmv, assuming equilibrium at 25 °C) (Knief and Dunfield 2005; Baani and Liesack 2008). Most, if not all, sources of CH_4, e.g., rice paddy soils, animal husbandry storage tank, and animal barn housings, emit CH_4 at concentrations in the order of several hundred ppmv at the maximum, as CH_4 diffuses rapidly after emission (Yoon et al. 2009). These concentrations are rarely high enough to ensure sustained CH_4 oxidation by the conventional methanotrophs (Knief and Dunfield 2005). A potential solution to this dilemma is to establish and maintain high-affinity CH_4-oxidizing population in the bioreactors (Yoon et al. 2009). The existence of methanotrophs capable of oxidizing CH_4 at concentrations as low as the atmospheric concentration (1.8 ppmv) has been suggested since the turn of the century (Bull et al. 2000). The analyses of *pmoA* genes of upland soils with atmospheric CH_4 sink capability identified the USCα (upland soil cluster α) clade of alphaproteobacterial methanotrophs with supposedly high affinity to CH_4 (Kolb et al. 2005). The genomic potential of the USCα methanotroph has recently been characterized using a targeted cell sorting approach coupled to metagenomic analysis (Pratscher et al. 2018). Evidences of high-affinity CH_4 oxidation were also found in

reports of experiments performed with pure cultures of alphaproteobacterial methanotrophs. Upon starvation, *Methylocystis* sp. LR1 exhibited half saturation constants as low as ~300 nM (equivalent to a gaseous concentration of ~210 ppmv at 25 °C) and *Methylocystis* strain SC2, possessing two dissimilar *pmoCAB* operons, was observed to oxidize CH_4 with a $K_{m,app}$ value of 0.11 μM with the expression and activity of higher-affinity pMMO2 (Dunfield and Conrad 2000; Baani and Liesack 2008). The wildtype *Methylocystis* strain SC2 was able to sustain its viability on CH_4 concentration as low as the atmospheric concentration, suggesting its potential utility in CH_4 emission mitigation. These experimental evidences should not be ignored despite the recently casted doubts on the existence of methanotrophs specifically suited for consumption of atmospheric CH_4 (Cai et al. 2016). There is no doubt that better understanding of physiology of such high-affinity methanotrophs and the ability to wield these organisms in engineered systems are crucial for successful development of practical biological CH_4 emission control strategies.

5 Climate-Smart Soils and Methanotrophs

The increasing demand for food, feed, and fibres to meet the global human population growth necessitates a sustainable circular economy to combat climate change. Meeting the global demands for these commodities entails conversion of native to arable lands and intensification of agriculture. Land-use related GHG emissions, including conversion to agricultural usage, account for approximately 25% of the global anthropogenic GHG emissions (Ciais et al. 2013; Tubiello et al. 2015). Climate-smart organic-based fertilization is one of the emerging GHG emission reduction strategies to offset the land-use related GHG emissions. In this approach, bio-based residues from various waste streams (e.g., livestock manure, plant waste materials, and anaerobic digester effluents) are recycled as soil additives to minimize GHG (mostly CH_4 and N_2O) emissions in agricultural soils, while sustaining or improving soil fertility, quality, and carbon storage capacity (Cayuela et al. 2010; Paustian et al. 2016). Recent research results suggest that optimization of the choice and combination of the residue materials may enable further reduction (Cayuela et al. 2010; Ho et al. 2017; Brenzinger et al. 2018).

The incorporation of bio-based residues in agricultural soils alters the abundance and composition of indigenous microbial populations and interactions among soil microorganisms (Hartmann et al. 2014; Sengupta and Dick 2015). Such shift in the microbial community composition and abundances may affect the methanotrophic population and the overall net methane emissions (Ho et al. 2015; Malghani et al. 2016). Upland agricultural soils are generally regarded as a relatively weak sink for atmospheric methane, when compared to native upland soils (Maxfield et al. 2008; Levine et al. 2011; Ho et al. 2015; Tate 2015; Malghani et al. 2016). However, the potential for methane oxidation in agricultural soils can be stimulated by the addition of specific organic residues (e.g., compost), thereby augmenting the methane sink function (Ho et al. 2015, 2019). Although the stimulatory effect was transient (20–30

days), the elevated methane uptake rate was comparable to those of native upland soils and could offset up to 16% of total GHG emissions, bringing into question whether repeated compost amendment in agricultural soils may or may not be able to sustain higher methane uptake rates for prolonged periods (Ho et al. 2015). Nevertheless, crop (common wheat) yield remained unchanged, suggesting that compost amendment was neither particularly beneficial nor detrimental to crop growth (Ho et al. 2017). Although slow mineralization rate of composted organics allow long-term deposition of organic carbon in the soil, compost is often deprived of nutrients and thus, is less beneficial in terms of plant growth enhancement (Ryals et al. 2015). A recent research suggested that the trade-off between higher crop yields and lower CH_4 emission may be reached considering the combination of compost and nutrient-rich bio-based residues, e.g., sewage sludge (Brenzinger et al. 2018). Organic amendments may also be combined with specific beneficial microorganisms (e.g., methanotrophs) to further improve the soil CH_4 uptake, as well as for promoting plant growth and suppressing plant pathogens (Berg 2009; Singh and Strong 2016; Carrión et al. 2018). Therefore, combinations of bio-based residues and microbial inocula at the optimum ratios to improve fertilization efficiency while curbing GHG emissions would be a potentially viable strategy to be implemented in climate-smart agriculture.

6 Harnessing the Methanotroph Interactome for Environmental Applications

Methanotrophs are typically employed as pure cultures for biotechnological applications, fueling the development of novel isolation strategies (Svenning et al. 2003; Kim et al. 2018; Kwon et al. 2018). In the environment, however, microorganisms rarely live in seclusion and methanotrophs are not exceptions. Soil and aquatic microorganisms co-exist and interact in the environment to modulate Earth's nutrient cycles. The response of methanotrophs to physico-chemical environmental parameters are widely documented; however, the interactions of methanotrophs with the biotic components of the Earth's environments are known to a lesser extent (Semrau et al. 2010; Ho et al. 2013). The investigations of methanotroph interactomes (i.e., mathanotrophs and accompanying non-methanotrophic community members) for environmental applications have, in fact, just recently gained scientific attention (Ho et al. 2016).

Several examples of direct and indirect modes of interactions constituting methanotroph interactomes can be found in recent literature (Fig. 3). A recent study suggested that methanotrophs may benefit from interaction with non-methanotrophic heterotrophs (e.g., *Rhizobium* spp.) that can provide the methanotrophs with the essential micronutrient vitamin B_{12} (Fig. 3a) (Iguchi et al. 2011). Co-culturing of *Methylovulum miyakonense* strain HT12 with the vitamin B_{12}-producing *Rhizobium* spp. stimulated CH_4 uptake and cell growth. In another recent work, co-habitation with methylotroph was found to influence the regulation of methanol dehydrogenase

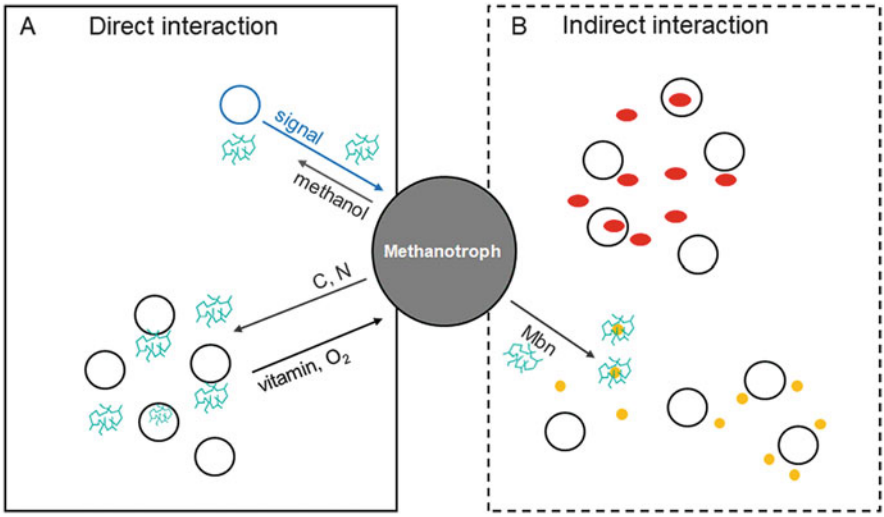

Fig. 3 Schematic representation of direct (**a**) and indirect (**b**) interactions within methanotroph interactomes. The empty circles of different colors denote non-methanotrophs. Indirect interactions represented in the panel (**a**), the blue circle and arrow depict chemical signaling of methylotrophs inducing methanol release from methanotrophs (Krause et al. 2017), whereas the black circle and arrow depict the flux of essential nutrients, e.g. vitamin B_{12}, and oxygen by other non-methanotrophs to the methanotrophs. In return, the methanotrophs provide assimilable carbon and nitrogen sources to the neighboring non-methanotrophs. In indirect interactions represented in the panel (**b**), release of methanobactin (Mbn, in green color) by methanotroph is depicted, which may impose a monopoly over bioavailable copper (solid yellow circles), effectively suppressing other microbial processes catalyzed by copper-dependent enzymes (Chang et al. 2018). Other non-methanotrophs may competitively uptake necessary micronutrients (solid red circles), which may, likewise, bar utilization by methanotrophs

in a gammaproteobacterial methanotroph, *Methylobacter tundripalodum* strain 31/32, inducing methanol release from the methanotroph (Krause et al. 2017). As a result, the methylotrophs in the co-culture thrived with methanotrophs despite the absence of non-CH_4 organic carbon substances in the medium. Conversely, as already mentioned above, *M. trichosporium* strain OB3b, and possibly most alphaproteobacterial methanotrophs with capability to synthesize methanobactin, may indirectly suppress other soil microbial processes such as N_2O reduction via secretion of methanobactin, imposing a 'copper monopoly' which depletes bioavailable copper needed for other cuproenzymes (Fig. 3b) (Chang et al. 2018).

Taking such interactome approach enables stable methanotrophic growth under conditions previously known to be unfit for methanotroph cultivation. Aerobic methanotrophs and strictly anaerobic methanogens conventionally thrive under seemingly contrasting conditions; however, a recent study have shown tight spatial organization of these microorganisms in a membrane bioreactor provided with acetate as the only substrate and maintained under oxygen-limiting condition. The methanogens provided CH_4 to the methanotrophs and the methanotrophs relieved O_2 tension in the microenvironments in return, to allow for survival of methanogens ('t Zandt et al.

2018). Driven by the incentives to generate higher value bio-products from biogas, van der Ha et al. (2012) successfully established stable enriched co-culture of methanotrophs with eukaryotic microalgae on biogas containing CH_4 and CO_2 but not O_2. Interestingly, such interactions between methanotrophs and non-methanotrophs may even occur in the presence of a physical barrier between the organisms with a cocktail of volatile organic compounds as the mediator of the interactome (Veraart et al. 2018). These studies exemplify diverse modes of biotic interactions within the methanotroph interactome at multi-trophic levels to modulate methanotrophic activity and cell growth.

Working with mixed microbial cultures, capitalizing on such interactions, often enhances growths and activities of the microorganisms within, and/or eliminates the necessity to operate under sterile conditions, lowering production costs (Ho et al. 2014; Strong et al. 2016). Methanotrophs, interacting with heterotrophs also enable development of environmental applications that are not feasible with pure methanotroph cultures. The association of aerobic methanotrophs and denitrifiers have been capitalized for developing nitrogen removal processes with CH_4 as the sole external carbon source (Eisentraeger et al. 2001; Modin et al. 2007; Zhu et al. 2016). A mixed community of methanotrophs and heterotrophs growing on organic substrates 'leaked' from the methanotrophs was able to collectively degrade a broad spectrum of micropollutants, a feat that could not be achieved with pure cultures of methanotrophs (Benner et al. 2015). Although still at its infancy, these studies provide an impetus for harnessing the methanotroph interactome for environmental applications. In future studies, further investigation of the key drivers regulating the methanotroph interactome is warranted for optimizing the intricate balances between the co-existing microorganisms to improve the effectiveness, stability, and reproducibility of the artificial communities engineered for specific environmental applications, as well as for devising novel environmental processes (Hays et al. 2015).

7 Conclusion and Future Perspective

Methanotrophs have now been studied for over 100 years since their first discovery, but continue to offer surprises. The new scientific discoveries regarding methanotroph physiology and ecology in the past few decades, e.g., copper regulation of MMOs, synthesis and utilization of methanobactin, high-affinity methane oxidation, and the discovery of the NC-10 denitrifying methanotrophs capable of generating O_2 internally from dismutation of NO, were among the most exciting discoveries in the field of microbiology and have had substantial implications in the development of novel environmental biotechnologies. As reviewed in this chapter, utilization of MMO-utilizing methanotrophs has already been vigorously studied for their practical engineering applications. Nevertheless, the new scientific discoveries are continuing to expand the horizon.

The recent discovery of the denitrifying anaerobic methanotrophs opened up new opportunities for improving sustainability of wastewater treatment processes (Shi et al. 2013; van Kessel et al. 2018). Combining anaerobic ammonia oxidation

(anammox) and denitrifying anaerobic methane oxidation (DAMO) by both archaea and *Methylomirabilis*-like bacteria in a laboratory-scale membrane biofilm reactor enabled simultaneous removal of NO_3^- and NH_4^+ without aeration (Luesken et al. 2011; Shi et al. 2013). The DAMO reactions may also be utilized for simultaneous oxidation of residual CH_4 and nitrogenous nutrients in wastewater treatment plant effluents (van Kessel et al. 2018). Likewise, the discoveries of the extremely acidophilic methanotrophs of the *Verrucomicrobia* phylum may lead to development of environmental cleanup technologies for extreme acidic environments utilizing the cometabolic activity of the pMMO expressed by these organisms (Semrau 2011). The collaborative efforts across disciplines are necessary, for bridging these hypothetical or nascent technologies to actual field applications. Better scientific understanding of the novel physiologies, capitalizing on the recent advances in molecular techniques, sequencing technology, and cell-imaging techniques, would be crucial for effectively wielding these microorganisms of great potential utility. Designing of innovative bioreactor configurations or field application methods for establishing and sustaining stable populations of these unconventional culture-resistant organisms and harnessing them with minimum energy consumption and greenhouse gas emissions would require rigorous efforts from the engineering side. As such, the relentless collaborative efforts of environmental microbiologists, microbial ecologists, and environmental and chemical engineers will be critical for success of future research on environmental applications of methanotrophs.

Acknowledgement This work was supported by the C1 Gas Refinery Program through the National Research Foundation of Korea (NRF), which is funded by the Ministry of Science, ICT and Future Planning (grant number: NRF-2015-M3D3A1A01064881) and the Deutsche Forschungsgemeinschaft (grant number: HO6234/1-1) and the Leibniz Universität Hannover.

References

Alvarez RA, Pacala SW, Winebrake JJ, Chameides WL, Hamburg SP (2012) Greater focus needed on methane leakage from natural gas infrastructure. Proc Nat Acad Sci 109:6435–6440

Alvarez-Cohen L, McCarty PL (1991) Product toxicity and cometabolic competitive inhibition modeling of chloroform and trichloroethylene transformation by methanotrophic resting cells. Appl Environ Microbiol 57:1031–1037

Alvarez-Cohen L, Speitel GE (2001) Kinetics of aerobic cometabolism of chlorinated solvents. Biodegradation 12:105–126

Amos BK, Ritalahti KM, Cruz-Garcia C, Padilla-Crespo E, Loffler FE (2008) Oxygen effect on *Dehalococcoides* viability and biomarker quantification. Environ Sci Technol 42:5718–5726

Anderson JE, McCarty PL (1997) Transformation yields of chlorinated ethenes by a methanotrophic mixed culture expressing particulate methane monooxygenase. Appl Environ Microbiol 63:687–693

Baani M, Liesack W (2008) Two isozymes of particulate methane monooxygenase with different methane oxidation kinetics are found in *Methylocystis* sp. strain SC2. Proc Nat Acad Sci 105:10203–10208

Baral BS, Bandow NL, Vorobev A, Freemeier BC, Bergman BH, Herdendorf TJ, Fuentes N, Ellias L, Turpin E, Semrau JD, DiSpirito AA (2014) Mercury binding by methanobactin from *Methylocystis* strain SB2. J Inorg Biochem 141:161–169

Benner J, De Smet D, Ho A, Kerckhof F-M, Vanhaecke L, Heylen K, Boon N (2015) Exploring methane-oxidizing communities for the co-metabolic degradation of organic micropollutants. Appl Microbiol Biotechnol 99:3609–3618

Berg G (2009) Plant–microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. Appl Microbiol Biotechnol 84:11–18

Brenzinger K, Drost SM, Korthals G, Bodelier PLE (2018) Organic residue amendments to modulate greenhouse gas aes from agricultural soils. Front Microbiol 9:3035

Brockman FJ, Payne W, Workman DJ, Soong A, Manley S, Hazen TC (1995) Effect of gaseous nitrogen and phosphorus injection on *in situ* bioremediation of a trichloroethylene-contaminated site. J Hazard Mater 41:287–298

Brown K, Tegoni M, Prudêncio M, Pereira AS, Besson S, Moura JJ, Moura I, Cambillau C (2000) A novel type of catalytic copper cluster in nitrous oxide reductase. Nat Struct Mol Biol 7:191–195

Bull ID, Parekh NR, Hall GH, Ineson P, Evershed RP (2000) Detection and classification of atmospheric methane oxidizing bacteria in soil. Nature 405:175–178

Cai Y, Zheng Y, Bodelier PLE, Conrad R, Jia Z (2016) Conventional methanotrophs are responsible for atmospheric methane oxidation in paddy soils. Nat Commun 7:11728

Carrión VJ, Cordovez V, Tyc O, Etalo DW, de Bruijn I, de Jager VCL, Medema MH, Eberl L, Raaijmakers JM (2018) Involvement of *Burkholderiaceae* and sulfurous volatiles in disease-suppressive soils. ISME J 12:2307–2321

Cayuela ML, Oenema O, Kuikman PJ, Bakker RR, van Groenigen JW (2010) Bioenergy by-products as soil amendments? Implications for carbon sequestration and greenhouse gas emissions. Glob Change Biol Bioenergy 2:201–213

Chang J, Gu W, Park D, Semrau JD, DiSpirito AA, Yoon S (2018) Methanobactin from *Methylosinus trichosporium* OB3b inhibits N_2O reduction in denitrifiers. ISME J 12:2086–2089

Chen Y, Dumont MG, Cébron A, Murrell JC (2007) Identification of active methanotrophs in a landfill cover soil through detection of expression of 16S rRNA and functional genes. Environ Microbiol 9:2855–2869

Chen Y, Dumont MG, McNamara NP, Chamberlain PM, Bodrossy L, Stralis-Pavese N, Murrell JC (2008) Diversity of the active methanotrophic community in acidic peatlands as assessed by mRNA and SIP-PLFA analyses. Environ Microbiol 10:446–459

Ciais P, Sabine C, Bala G, Bopp L, Brovkin V, Canadell J, Chhabra A, DeFries R, Galloway J, Heimann M, Jone C, Quéré CL, Myneni RB, Piao S, Thornton P (2013) Carbon and other biogeochemical cycles. In: Stocker TF, Qin D, Plattner G-K, Tignor M, Allen SK, Boschung J et al (eds) Climate change 2013: the physical science basis contribution of working group I to the fifth assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge, pp 465–570

DiSpirito AA, Semrau JD, Murrell JC, Gallagher WH, Dennison C, Vuilleumier S (2016) Methanobactin and the link between copper and bacterial methane oxidation. Microbiol Mol Biol Rev 80:387–409

Duba AG, Jackson KJ, Jovanovich MC, Knapp RB, Taylor RT (1996) TCE remediation using *in situ*, resting-state bioaugmentation. Environ Sci Technol 30:1982–1989

Dunfield PF, Conrad R (2000) Starvation alters the apparent half-saturation constant for methane in the type II methanotroph *Methylocystis* strain LR1. Appl Environ Microbiol 66:4136–4138

Eguchi M, Kitagawa M, Suzuki Y, Nakamuara M, Kawai T, Okamura K, Sasaki S, Miyake Y (2001) A field evaluation of *in situ* biodegradation of trichloroethylene through methane injection. Water Res 35:2145–2152

Eisentraeger A, Klag P, Vansbotter B, Heymann E, Dott W (2001) Denitrification of groundwater with methane as sole hydrogen donor. Water Res 35:2261–2267

Elango NA, Radhakrishnan R, Froland WA, Wallar BJ, Earhart CA, Lipscomb JD, Ohlendorf DH (1997) Crystal structure of the hydroxylase component of methane monooxygenase from *Methylosinus trichosporium* OB3b. Protein Sci 6:556–568

Forrester SB, Han J-I, Dybas MJ, Semrau JD, Lastoskie CM (2005) Characterization of a mixed methanotrophic culture capable of chloroethylene degradation. Environ Eng Sci 22:178–186

Fox BG, Froland WA, Dege JE, Lipscomb JD (1989) Methane monooxygenase from *Methylosinus trichosporium* OB3b. Purification and properties of a three-component system with high specific activity from a type II methanotroph. J Biol Chem 264:10023–10033

Ganendra G, De Muynck W, Ho A, Hoefman S, De Vos P, Boeckx P, Boon N (2014) Atmospheric methane removal by methane-oxidizing bacteria immobilized on porous building materials. Appl Microbiol Biotechnol 98:3791–3800

Ganendra G, Mercado-Garcia D, Hernandez-Sanabria E, Peiren N, De Campeneere S, Ho A, Boon N (2015) Biofiltration of methane from ruminants gas effluent using autoclaved aerated concrete as the carrier material. Chem Eng J 277:318–323

Gebert J, Gröngröft A (2006) Performance of a passively vented field-scale biofilter for the microbial oxidation of landfill methane. Waste Manage 26:399–407

Gebert J, Gröengröeft A, Miehlich G (2003) Kinetics of microbial landfill methane oxidation in biofilters. Waste Manage 23:609–619

Gebert J, Gröngröft A, Schloter M, Gattinger A (2004) Community structure in a methanotroph biofilter as revealed by phospholipid fatty acid analysis. FEMS Microbiol Lett 240:61–68

Godfray HCJ, Beddington JR, Crute IR, Haddad L, Lawrence D, Muir JF, Pretty J, Robinson S, Thomas SM, Toulmin C (2010) Food security: the challenge of feeding 9 billion people. Science 327:812–818

Han J-I, Lontoh S, Semrau JD (1999) Degradation of chlorinated and brominated hydrocarbons by *Methylomicrobium album* BG8. Arch Microbiol 172:393–400

Hanson RS, Hanson TE (1996) Methanotrophic bacteria. Microbiol Rev 60:439–471

Hartmann M, Frey B, Mayer J, Mäder P, Widmer F (2014) Distinct soil microbial diversity under long-term organic and conventional farming. ISME J 9:1177–1194

Hays SG, Patrick WG, Ziesack M, Oxman N, Silver PA (2015) Better together: engineering and application of microbial symbioses. Curr Opin Biotechnol 36:40–49

Hazen TC, Looney BB, Enzien M, Franck MM, Fliemans CB, Eddy CA (1993) *In-situ* bioremediation via horizontal wells. I&EC Special Symposium, American Chemical Society, Atlanta, GA

Hazen TC, Chakraborty R, Fleming JM, Gregory IR, Bowman JP, Jimenez L, Zhang D, Pfiffner SM, Brockman FJ, Sayler GS (2009) Use of gene probes to assess the impact and effectiveness of aerobic *in situ* bioremediation of TCE. Arch Microbiol 191:221–232

He J, Ritalahti KM, Aiello MR, Löffler FE (2003a) Complete detoxification of vinyl chloride by an anaerobic enrichment culture and identification of the reductively dechlorinating population as a *Dehalococcoides* species. Appl Environ Microbiol 69:996–1003

He J, Ritalahti KM, Yang K-L, Koenigsberg SS, Löffler FE (2003b) Detoxification of vinyl chloride to ethene coupled to growth of an anaerobic bacterium. Nature 424:62–65

Ho A, Kerckhof F-M, Luke C, Reim A, Krause S, Boon N, Bodelier PLE (2013) Conceptualizing functional traits and ecological characteristics of methane-oxidizing bacteria as life strategies. Environ Microbiol Rep 5:335–345

Ho A, de Roy K, Thas O, De Neve J, Hoefman S, Vandamme P, Heylen K, Boon N (2014) The more, the merrier: heterotroph richness stimulates methanotrophic activity. ISME J 8:1945–1948

Ho A, Reim A, Kim SY, Meima-Franke M, Termorshuizen A, de Boer W, van der Putten WH, Bodelier PL (2015) Unexpected stimulation of soil methane uptake as emergent property of agricultural soils following bio-based residue application. Glob Change Biol 21:3864–3879

Ho A, Angel R, Veraart AJ, Daebeler A, Jia Z, Kim SY, Kerckhof F-M, Boon N, Bodelier PLE (2016) Biotic interactions in microbial communities as modulators of biogeochemical processes: methanotrophy as a model system. Front Microbiol 7:1285

Ho A, Ijaz UZ, Janssens TKS, Ruijs R, Kim SY, de Boer W, Termorshuizen A, van der Putten WH, Bodelier PLE (2017) Effects of bio-based residue amendments on greenhouse gas emission from agricultural soil are stronger than effects of soil type with different microbial community composition. Glob Change Biol Bioenergy 9:1707–1720

Ho A, Mo Y, Lee HJ, Sauheitl L, Jia Z, Horn MA (2018) Effect of salt stress on aerobic methane oxidation and associated methanotrophs; a microcosm study of a natural community from a non-saline environment. Soil Biol Biochem 125:210–214

Ho A, Lee HJ, Reumer M, Meima-Franke M, Raaijmakers C, Zweers H, de Boer W, can der Putten WH, Bodelier PLE (2019) Unexpected role of canonical aerobic methanotrophs in upland agricultural soils. Soil Biol Biochem 131:1–8

Holliger C, Wohlfarth G, Diekert G (1998) Reductive dechlorination in the energy metabolism of anaerobic bacteria. FEMS Microbiol Rev 22:383–398

Hou Y, Velthof GL, Oenema O (2015) Mitigation of ammonia, nitrous oxide and methane emissions from manure management chains: a meta-analysis and integrated assessment. Global Change Biol 21:1293–1312

Hulse CL, Tiedje JM, Averill BA (1989) Evidence for a copper-nitrosyl intermediate in denitrification by the copper-containing nitrite reductase of *Achromobacter cycloclastes*. J Am Chem Soc 111:2322–2323

Iguchi H, Yurimoto H, Sakai Y (2011) Stimulation of methanotrophic growth in cocultures by cobalamin excreted by rhizobia. Appl Environ Microbiol 77:8509–8515

in 't Zandt MH, van den Bosch TJM, Rijkers R, van Kessel MAHJ, MSM J, Welte CU (2018) Co-cultivation of the strictly anaerobic methanogen *Methanosarcina barkeri* with aerobic methanotrophs in an oxygen-limited membrane bioreactor. Appl Microbiol Biotechnol 102:5685–5694

Islam T, Torsvik V, Larsen Ø, Bodrossy L, Øvreås L, Birkeland N-K (2016) Acid-tolerant moderately thermophilic methanotrophs of the class *Gammaproteobacteria* isolated from tropical topsoil with methane seeps. Front Microbiol 7:851

Jiang H, Chen Y, Jiang P, Zhang C, Smith TJ, Murrell JC, Xing X-H (2010) Methanotrophs: multifunctional bacteria with promising applications in environmental bioengineering. Biochem Eng J 49:277–288

Keck J, Sims RC, Coover M, Park K, Symons B (1989) Evidence for cooxidation of polynuclear aromatic hydrocarbons in soil. Water Res 23:1467–1476

Kenney GE, Sadek M, Rosenzweig AC (2016) Copper-responsive gene expression in the methanotroph *Methylosinus trichosporium* OB3b. Metallomics 8:931–940

Khadka R, Clothier L, Wang L, Lim CK, Klotz MG, Dunfield PF (2018) Evolutionary history of copper membrane monooxygenases. Front Microbiol 9:2493

Kim HJ, Graham DW, DiSpirito AA, Alterman MA, Galeva N, Larive CK, Asunskis D, Sherwood PMA (2004) Methanobactin, a copper-acquisition compound from methane-oxidizing bacteria. Science 305:1612–1615

Kim J, Kim DD, Yoon S (2018) Rapid isolation of fast-growing methanotrophs from environmental samples using continuous cultivation with gradually increased dilution rates. Appl Microbiol Biotechnol 102:5707–5715

Kip N, Ouyang W, van Winden J, Raghoebarsing A, van Niftrik L, Pol A, Pan Y, Bodrossy L, van Donselaar EG, Reichart GJ, Jetten MS, Damsté JS, Op den Camp HJ (2011) Detection, isolation, and characterization of acidophilic methanotrophs from sphagnum mosses. Appl Environ Microbiol 77:5643–5654

Kittelmann S, Friedrich MW (2008) Novel uncultured *Chloroflexi* dechlorinate perchloroethene to trans-dichloroethene in tidal flat sediments. Environ Microbiol 10:1557–1570

Knief C, Dunfield PF (2005) Response and adaptation of different methanotrophic bacteria to low methane mixing ratios. Environ Microbiol 7:1307–1317

Knittel K, Boetius A (2009) Anaerobic oxidation of methane: progress with an unknown process. Annu Rev Microbiol 63:311–334

Kolb S, Knief C, Dunfield PF, Conrad R (2005) Abundance and activity of uncultured methanotrophic bacteria involved in the consumption of atmospheric methane in two forest soils. Environ Microbiol 7:1150–1161

Krause SMB, Johnson T, Samadhi Karunaratne Y, Fu Y, Beck DAC, Chistoserdova L, Lidstrom ME (2017) Lanthanide-dependent cross-feeding of methane-derived carbon is linked by microbial community interactions. Proc Nat Acad Sci 114:358–363

Kumaresan D, Stephenson J, Doxey AC, Bandukwala H, Brooks E, Hillebrand-Voiculescu A, Whiteley AS, Murrell JC (2018) Aerobic proteobacterial methylotrophs in Movile Cave: genomic and metagenomic analyses. Microbiome 6:1

Kwon M, Ho A, Yoon S (2018) Novel approaches and reasons to isolate methanotrophic bacteria with biotechnological potentials: recent achievements and perspectives. Appl Microbiol Biotechnol 103:1–8

La H, Hettiaratchi JPA, Achari G, Dunfield PF (2018) Biofiltration of methane. Bioresour Technol 268:759–772

Leahy JG, Batchelor PJ, Morcomb SM (2003) Evolution of the soluble diiron monooxygenases. FEMS Microbiol Rev 27:449–479

Lee S-W, Keeney DR, Lim D-H, Dispirito AA, Semrau JD (2006) Mixed pollutant degradation by *Methylosinus trichosporium* OB3b expressing either soluble or particulate methane monooxygenase: can the tortoise beat the hare? Appl Environ Microbiol 72:7503–7509

Lee S-W, Im J, DiSpirito AA, Bodrossy L, Barcelona MJ, Semrau JD (2009) Effect of nutrient and selective inhibitor amendments on methane oxidation, nitrous oxide production, and key gene presence and expression in landfill cover soils: characterization of the role of methanotrophs, nitrifiers, and denitrifiers. Appl Microbiol Biotechnol 85:389–403

Levine UY, Teal TK, Robertson GP, Schmidt TM (2011) Agriculture's impact on microbial diversity and associated fluxes of carbon dioxide and methane. ISME J 5:1683–1691

Lichtmannegger J, Leitzinger C, Wimmer R, Schmitt S, Schulz S, Kabiri Y, Eberhagen C, Rieder T, Janik D, Neff F, Straub BK, Schirmacher P, DiSpirito AA, Bandow N, Baral BS, Flately A, Kremmer E, Denk G, Reiter FP, Hohenester S, Eckardt-Schupp F, Dencher NA, Adamski J, Sauer V, Niemietz C, Schmidt HH, Merle U, Gotthardt DN, Kroemer G, Weiss KH, Zischka H (2016) Methanobactin reverses acute liver failure in a rat model of Wilson disease. J Clin Invest 126:2721–2735

Lieberman RL, Rosenzweig AC (2005) Crystal structure of a membrane-bound metalloenzyme that catalyses the biological oxidation of methane. Nature 434:177

Liebner S, Svenning MM (2013) Environmental transcription of *mmoX* by methane-oxidizing *Proteobacteria* in a subarctic palsa peatland. Appl Environ Microbiol 79:701–706

Limbri H, Gunawan C, Thomas T, Smith A, Scott J, Rosche B (2014) Coal-packed methane biofilter for mitigation of greenhouse gas emissions from coal mine ventilation air. PLoS One 9:e94641

Löffler FE, Ritalahti KM, Zinder SH (2013) *Dehalococcoides* and reductive dechlorination of chlorinated solvents. In: Stroo HF, Leeson A, Ward CH (eds) Bioaugmentation for groundwater remediation. Springer, New York, pp 39–88

Lontoh S, Semrau JD (1998) Methane and trichloroethylene degradation by *Methylosinus trichosporium* OB3b expressing particulate methane monooxygenase. Appl Environ Microbiol 64:1106–1114

Lontoh S, DiSpirito AA, Semrau JD (1999) Dichloromethane and trichloroethylene inhibition of methane oxidation by the membrane-associated methane monooxygenase of *Methylosinus trichosporium* OB3b. Arch Microbiol 171:301–308

Lu X, Gu W, Zhao L, Farhan Ul Haque M, DiSpirito AA, Semrau JD, Gu B (2017) Methylmercury uptake and degradation by methanotrophs. Sci Adv 3:e1700041

Luesken FA, Sánchez J, van Alen TA, Sanabria J, Op den Camp HJM, Jetten MSM, Kartal B (2011) Simultaneous nitrite-dependent anaerobic methane and ammonium oxidation processes. Appl Environ Microbiol 19:6802–6807

Malghani S, Reim A, von Fischer J, Conrad R, Kuebler K, Trumbore SE (2016) Soil methanotroph abundance and community composition are not influenced by substrate availability in laboratory incubations. Soil Biol Biochem 101:184–194

Matturro B, Presta E, Rossetti S (2016) Reductive dechlorination of tetrachloroethene in marine sediments: biodiversity and dehalorespiring capabilities of the indigenous microbes. Sci Total Environ 545–546:445–452

Maxfield PJ, Hornibrook ERC, Evershed RP (2008) Acute impact of agriculture on high-affinity methanotrophic bacterial populations. Environ Microbiol 10:1917–1924

Melse RW, van der Werf AW (2005) Biofiltration for mitigation of methane emission from animal husbandry. Environ Sci Technol 39:5460–5468

Modin O, Fukushi K, Yamamoto K (2007) Denitrification with methane as external carbon source. Water Res 41:2726–2738

Murrell JC, McDonald IR, Gilbert B (2000) Regulation of expression of methane monooxygenases by copper ions. Trend Microbiol 8:221–225

Nikiema J, Bibeau L, Lavoie J, Brzezinski R, Vigneux J, Heitz M (2005) Biofiltration of methane: an experimental study. Chem Eng J 113:111–117

Oldenhuis R, Oedzes JY, van der Waarde JJ, Janssen DB (1991) Kinetics of chlorinated hydrocarbon degradation by *Methylosinus trichosporium* OB3b and toxicity of trichloroethylene. Appl Environ Microbiol 57:7–14

Op den Camp HJM, Islam T, Stott MB, Harhangi HR, Hynes A, Schouten S, Jetten MS, Birkeland NK, Pol A, Dunfield PF (2009) Environmental, genomic and taxonomic perspectives on methanotrophic *Verrucomicrobia*. Environ Microbiol Rep 1:293–306

Park S, Ely RL (2008) Candidate stress genes of *Nitrosomonas europaea* for monitoring inhibition of nitrification by heavy metals. Appl Environ Microbiol 74:5475–5482

Paustian K, Lehmann J, Ogle S, Reay D, Robertson GP, Smith P (2016) Climate-smart soils. Nature 532:49–57

Popat SC, Deshusses MA (2009) Reductive dehalogenation of trichloroethene vapors in an anaerobic biotrickling filter. Environ Sci Technol 43:7856–7861

Pratscher J, Vollmers J, Wiegand S, Dumont MG, Kaster A-K (2018) Unravelling the identity, metabolic potential and global biogeography of the atmospheric methane-oxidizing upland soil cluster α. Environ Microbiol 20:1016–1029

Pratt C, Walcroft AS, Tate KR, Ross DJ, Roy R, Reid MH, Veiga PW (2012) Biofiltration of methane emissions from a dairy farm effluent pond. Agric Ecosyst Environ 152:33–39

Raghoebarsing AA, Pol A, van de Pas-Schoonen KT, Smolders AJP, Ettwig KF, Rijpstra WIC, Schouten S, Sinninghe Damsté JS, Op den Camp HJM, Jetten MSM, Strous M (2006) A microbial consortium couples anaerobic methane oxidation to denitrification. Nature 440:918

Rahman MT, Crombie A, Chen Y, Stralis-Pavese N, Bodrossy L, Meir P, McNamara NP, Murrell JC (2011) Environmental distribution and abundance of the facultative methanotroph *Methylocella*. ISME J 5:1061–1066

Ritalahti KM, Löffler FE, Rasch EE, Koenigsberg SS (2005) Bioaugmentation for chlorinated ethene detoxification: bioaugmentation and molecular diagnostics in the bioremediation of chlorinated ethene-contaminated sites. Ind Biotechnol 1:114–118

Ryals R, Hartman MD, Parton WJ, DeLonge MS, Silver WL (2015) Long-term climate change mitigation potential with organic matter management on grasslands. Ecol Appl 25:531–545

Scheutz C, Kjeldsen P, Bogner JE, De Visscher A, Gebert J, Hilger HA, Huber-Humer M, Spokas K (2009) Microbial methane oxidation processes and technologies for mitigation of landfill gas emissions. Waste Manage Res 27:409–455

Semprini L, McCarty PL (1991) Comparison between model simulations and field results for in-situ biorestoration of chlorinated aliphatics: Part 1. Biostimulation of methanotrophic bacteria. Groundwater 29:365–374

Semrau J (2011) Bioremediation via methanotrophy: overview of recent findings and suggestions for future research. Front Microbiol 2:209

Semrau J, DiSpirito A, Yoon S (2010) Methanotrophs and copper. FEMS Microbiol Rev 34:496–531

Sengupta A, Dick WA (2015) Bacterial community diversity in soil under two tillage practices as determined by pyrosequencing. Microbial Ecol 70:853–859

Shi Y, Hu S, Lou J, Lu P, Keller J, Yuan Z (2013) Nitrogen removal from wastewater by coupling anammox and methane-dependent denitrification in a membrane biofilm reactor. Environ Sci Technol 47:11577–11583

Singh JS, Strong PJ (2016) Biologically derived fertilizer: a multifaceted bio-tool in methane mitigation. Ecotoxicol Environ Saf 124:267–276

Streese J, Stegmann R (2003) Microbial oxidation of methane from old landfills in biofilters. Waste Manage 23:573–580

Strong P, Laycock B, Mahamud S, Jensen P, Lant P, Tyson G, Pratt S (2016) The opportunity for high-performance biomaterials from methane. Microorganisms 4:11

Svenning MM, Wartiainen I, Hestnes AG, Binnerup SJ (2003) Isolation of methane oxidising bacteria from soil by use of a soil substrate membrane system. FEMS Microbiol Ecol 44:347–354

Takeuchi M, Nanba K, Iwamoto H, Nirei H, Kusuda T, Kazaoka O, Owaki M, Furuya K (2005) *In situ* bioremediation of a *cis*-dichloroethylene-contaminated aquifer utilizing methane-rich groundwater from an uncontaminated aquifer. Water Res 39:2438–2444

Tate KR (2015) Soil methane oxidation and land-use change – from process to mitigation. Soil Biol Biochem 80:260–272

Taylor RT, Hanna ML, Shah NN, Shonnard DR, Duba AG, Durham WB, Jackson KJ, Knapp RB, Wijesinghe AM, Knezovich JP, Jovanovich MC (1993) *In situ* bioremediation of trichloroethylene-contaminated water by a resting-cell methanotrophic microbial filter. Hydrol Sci J 38:323–342

Tubiello FN, Salvatore M, Ferrara AF, House J, Federici S, Rossi S, Biancalani R, Condor Golec RD, Jacobs H, Flammini A, Prosperi P, Gardenas-Galindo P, Schmidhuber J, Sanz Sanchez MJ, Srivastava N, Smith P (2015) The contribution of agriculture, forestry and other land use activities to global warming, 1990–2012. Glob Chang Biol 21:2655–2660

van der Ha D, Nachtergaele L, Kerckhof F-M, Rameiyanti D, Bossier P, Verstraete W, Boon N (2012) Conversion of biogas to bioproducts by algae and methane oxidizing bacteria. Environ Sci Technol 46:13425–13431

van Hylckama Vlieg JET, de Koning W, Janssen DB (1996) Transformation kinetics of chlroinated ethenes by *Methylosinus trichosporium* OB3b and detection of unstable epoxides by on-line gas chromatography. Appl Environ Microbiol 62:3304–3312

van Hylckama Vlieg JET, Janssen DB (2001) Formation and detoxification of reactive intermediates in the metabolism of chlorinated ethenes. J Biotechnol 85:81–102

van Kessel MAHJ, Stultiens K, Slegers MFW, Guerrero Cruz S, Jetten MSM, Kartal B, Op den Camp HJM (2018) Current perspectives on the application of N-damo and anammox in wastewater treatment. Curr Opin Biotechnol 50:222–227

Veraart AJ, Garbeva P, van Beersum F, Ho A, Hordijk CA, Meima-Franke M, Zweers AJ, Bodelier PLE (2018) Living apart together – bacterial volatiles influence methanotrophic growth and activity. ISME J 12:1163–1166

Vorobev A, Jagadevan S, Baral BS, DiSpirito AA, Freemeier BC, Bergman BH, Bandow NL, Semrau JD (2013) Detoxification of mercury by methanobactin from *Methylosinus trichosporium* OB3b. Appl Environ Microbiol 79:5918–5926

Wendlandt K-D, Stottmeister U, Helm J, Soltmann B, Jechorek M, Beck M (2010) The potential of methane-oxidizing bacteria for applications in environmental biotechnology. Eng Life Sci 10:87–102

Wu YM, Yang J, Fan XL, Fu SF, Sun MT, Guo RB (2017) Elimination of methane in exhaust gas from biogas upgrading process by immobilized methane-oxidizing bacteria. Bioresour Technol 231:124–128

Yamazaki S, Koyama J, Miyake H (2014) Inventors; Semiconductor Energy Laboratory Co., Ltd., assignee. Semiconductor device having an oxide semiconductor with a crystalline region and manufacturing method thereof. United States patent US 8,633,480 B2

Yang Y, Cápiro NL, Yan J, Marcet TF, Pennell KD, Löffler FE (2017a) Resilience and recovery of *Dehalococcoides mccartyi* following low pH exposure. FEMS Microbiol Ecol 93:fix130

Yang Y, Higgins SA, Yan J, Simsir B, Chourey K, Iyer R, Hettich RL, Baldwin B, Oqles DM, Löffler FE (2017b) Grape pomace compost harbors organohalide-respiring Dehalogenimonas species with novel reductive dehalogenase genes. ISME J 11:2767–2780

Yoon S, Semrau JD (2008) Measurement and modeling of multiple substrate oxidation by methanotrophs at 20 °C. FEMS Microbiol Lett 287:156–162

Yoon S, Carey J, Semrau J (2009) Feasibility of atmospheric methane removal using methanotrophic biotrickling filters. Appl Microbiol Biotechnol 83:949–956

Zahn JA, DiSpirito AA (1996) Membrane-associated methane monooxygenase from *Methylococcus capsulatus* (Bath). J Bacteriol 178:1018–1029

Zakirov V, Sweeting M, Lawrence T, Sellers J (2001) Nitrous oxide as a rocket propellant. Acta Astronaut 48:353–362

Zhu J, Wang Q, Yuan M, Tan G-YA, Sun F, Wang C, Wu W, Lee PH (2016) Microbiology and potential applications of aerobic methane oxidation coupled to denitrification (AME-D) process: a review. Water Res 90:203–215