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ScienceDirect
Organic Geochemistry 37 (2006) 1411–1419
Organic Geochemistry
www.elsevier.com/locate/orggeochem

Methane dynamics in a microbial community of the Black Sea traced by stable carbon isotopes *in vitro*

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Received 8 November 2005; accepted 19 March 2006
Available online 6 June 2006

Abstract

Microbial mats thriving at gas seeps within anoxic bottom water were sampled during a Black Sea expedition in summer 2001. These mats contain associations of archaea which belong to the ANME-1 and, to a lesser extent, to the ANME-2 cluster, both accompanied by sulphate-reducing bacteria of the *Desulfosarcina/Desulfococcus*-group and perform the anaerobic oxidation of methane (AOM). Transferring living mat into the laboratory allowed for an *in vitro* investigation of its methane turnover. We incubated aliquots of these AOM performing mats over a time period of 242 days tracking concentrations and $^{13}C/^{12}C$ ratios of the methane. The data obtained showed a decrease in concentration accompanied by a relative enrichment of ^{12}C in the residual methane. These results indicate that the mats performed, in the presence of sulphate, both methane oxidation and methane production (methanogenesis). Rate calculations using information from additional experiments and field data gave a ratio between methane oxidation and methanogenesis of about 2:1. The parallel investigation of concentrations and signatures of stable carbon isotopes of methane revealed methane dynamics within these mats exceeding considerably what becomes apparent from only recognising the methane concentrations.

1. Introduction

Methane is known to be of particular importance in terms of the atmospheric chemistry and the natural and anthropogenic greenhouse effect. Moreover, several recent findings have added to the combined efforts to understand the biogeochemistry of methane: (i) the recognition of the existence of the anaerobic oxidation of methane (Barnes and Goldberg, 1976; Reeburgh, 1976) and the importance of this process to the global methane cycle by subsequent work, (ii) the discovery of vast amounts of methane in gas hydrates present within marine sediments and permafrost soils (Kvenvolden et al., 1993; Kvenvolden and Lorenson, 2001), (iii) the observation of substantial changes in the methane content of the atmosphere and its close relation to atmospheric

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doi:10.1016/j.orggeochem.2006.03.007

temperature records within the last 420,000 years (Petit et al., 1999; Wuebbles and Hayhoe, 2002), and (iv) indications for large-scale methane releases in the geological record causing substantial effects on the global atmosphere and hydrosphere (Dickens et al., 1995; Haq, 1998; Beerling and Berner, 2002; de Wit et al., 2002).

Stable carbon isotope determinations represent a well-established tool to trace transformation processes especially for compounds of low carbon numbers that show strong kinetic isotope effects.

Several studies were accomplished on the kinetic carbon isotope fractionation accompanying bacterial methane oxidation in oxygenated milieus, resulting in fractionation factors (αC) ranging between 1.003 and 1.037 (Silverman and Oyama, 1968; Barker and Fritz, 1981; Coleman et al., 1981; Tyler et al., 1994; Bergamaschi and Harris, 1995; Reeburgh et al., 1997).

Much less is known about the carbon isotope fractionation during the anaerobic oxidation of methane (AOM). A first study by Whiticar and Faber (1986) who applied three different calculation models on published data sets from marine and brackish pore water profiles yielded fractionation factors in the range of 1.002–1.014. Other calculations based on marine pore water data were done by Alperin et al. (1988) with αC values of 1.0088 ± 0.0013 and more recently by Martens et al. (1999) with slightly higher αC values of 1.011 ± 0.001. Grossman et al. (2002) obtained αC values of 1.0136 ± 0.001 from studies of a landfill-leachate contaminated aquifer. So far, no data from *in vitro* experiments are available. Though sulphate reduction coupled AOM and microbial methanogenesis have thought to be spatially mutually exclusive for a long time, recent work points to a conjoint presence of both processes in marine environments Pimenov et al., 1997; Hoehler et al., 2000; Inagaki et al., 2004; Krüger et al., 2003, 2005;). Moreover, there is evidence that the same organisms can be involved in both AOM and methanogenesis (Krüger et al., 2003; Hallam et al., 2004; Orcutt et al., 2005). However, the biochemical functioning of AOM and the physiology of the relevant microorganisms remain poorly understood.

Recent investigations have shown the massive occurrence of microbial mats in the Black Sea that thrive on methane and are mainly built by consortia of methanotrophic archaea and sulphate-reducing bacteria. Molecular microbiological and biomarker analysis revealed that the archaea are dominated by members of the ANME-1 cluster (Michaelis et al., 2002; Blumenberg et al., 2004; Treude et al., 2005).

We incubated pieces of a microbial mat performing AOM recovered from the Black Sea. During an incubation period of 242 days, we traced the concentration and the signature of stable carbon isotopes of methane to gain information on the turnover of methane.

2. Samples and methods

2.1. Samples

Mat samples were obtained during a research cruise with the RV 'Professor Logachev' in July 2001 from the anoxic waters of the NW Black Sea shelf (44°46.5′N, 31°59.6′E; water depth 230 m (Michaelis et al., 2002)). Mat pieces were taken with the manipulator of the submersible JAGO, placed into a barrel filled with anoxic *in situ* water, and the closed barrel was brought to the surface. On board, the samples were immediately transferred into glass bottles with anoxic Black Sea water, sealed with butyl-rubber stoppers and stored at 8 °C under an atmosphere of methane until further processing. A detailed characterisation of the microbial community in the mat used for the *in vitro* experiments was published previously by Blumenberg et al. (2004) based on lipid and molecular microbiological work (sample code C; resembling the sample described in Michaelis et al. (2002)). It was shown that, in addition to the clearly predominating ANME-1 archaea members of the ANME-2 cluster are also present, although to a much lesser extent (accompanied by specific sulphate-reducing bacteria [SRB] of the *Desulfosarcina/Desulfococcus* δ-proteobacteria subgroup). Moreover, an *in vitro* incubation with ^{13}C-labelled CH_4 of the mat used also for this study showed a higher uptake of methane carbon into lipids specific for ANME-1 compared to ANME-2 archaea (Blumenberg et al., 2005).

2.2. Experimental set up

Experiments for the measurement of methane consumption were carried out in glass vials (20 ml each) sealed with butyl-rubber stoppers and screw caps. Pieces of mat material were homogenised in a bowl. For each of three experiments (A, B, and C) conducted in parallel, 500 µl of this mat suspen-

sion were incubated with 10 ml artificial seawater medium (salinity and sulphate concentrations were adjusted to the respective *in situ* conditions of ~22‰ and 16 mM, respectively). The same set up was used for the control, but no mat suspension was added. All manipulations were performed under an atmosphere of N_2/CO_2 (90/10 [v/v]) in an anoxic glove chamber (MECAPLEX). The remaining headspace of 10 ml was filled with 50% CH_4 and 50% N_2/CO_2 (90/10 [v/v]). Thereafter, the bottles were incubated at 12 °C and gently shaken once per day horizontally to facilitate mixing of methane and sulphate. Headspace samples (100 µl) for analyses of CH_4 concentrations and $\delta^{13}C$–CH_4 were taken with gas-tight syringes at the beginning of the experiments and after 28, 59, 104, 166, and 242 days, respectively. For methane production rates, the same set up and medium were used. The headspace was flushed with N_2/CO_2 (90/10 [v/v]), and CH_4 concentrations were below the detection limit (<1 ppmv) 1 h after the start of incubation. Triplicate tubes were incubated for two weeks horizontally at 12 °C and gently shaken once per day to facilitate mixing of substrates and sulphate. As potential substrates for methanogenesis the following final concentrations were added: acetate (10 mM), formate (10 mM), methanol (5 mM), methylamines (10 mM), molecular hydrogen (80% v), and N_2/CO_2 (90/10 [v/v]) in control experiments. Headspace samples were taken daily with gas-tight syringes and analysed for CH_4 concentrations.

2.3. Analytical procedures

2.3.1. CH_4 concentrations

CH_4 concentrations were determined using a GC 14B gas chromatograph (Shimadzu) equipped with a Supel-Q Plot column (30 m × 0.53 mm; Supelco) and a flame ionisation detector. The carrier gas was N_2 at a flow rate of 3 ml min^{-1}. The column temperature was 110 °C.

2.3.2. Sulphide determinations

Sulphide was determined using the methylene blue assay (Aeckersberg et al., 1991).

2.3.3. Stable carbon isotope signatures of CH_4

$\delta^{13}C$ signatures were analysed using coupled gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS), using a Finnigan DeltaPlusXL mass spectrometer equipped with a HP 6890 gas chromatograph and CuO/Ni/Pt combustion furnace operated at 1010 °C. Gases were injected via an 8 Port Valco-valve into a carrier gas flow of 6 ml min^{-1}. The gases were trapped on a Gerstel Cooled Injection System 4 (CIS4) packed with 10 mg PoraPak Q (80–100 mesh) and operated in splitless mode at −135 °C for 8 min. The gases were desorbed through heating the injector to 60 °C at 12 °C s^{-1} and held at this temperature for 1.6 min. The gas chromatograph was equipped with a CP Plot molsieve 5 Å capillary column (25 m, 0.32 mm i.d., 30 µm film thickness), which ensures separation of CH_4 from N_2 at ambient temperatures (30 °C). Standard deviations for replicate injections were less than ±0.7‰. GC-C-IRMS precision was checked daily using a CH_4 standard with known isotopic composition. The carbon isotope ratios are expressed in a permil deviation from the Vienna Belemnite (VPDB) standard in the usual δ-notation:

$$\delta^{13}C = [(R_{sample}/R_{standard}) - 1] \cdot 1000$$

where R is the $^{13}C/^{12}C$ ratio and $R_{standard} = 0.0112372$.

Isotopic fractionation factors are expressed in the α-notation for a reaction $A \rightarrow B$, α is defined as

$$\alpha(AB) = (\delta A + 1000)/(\delta B + 1000)$$

where δA and δB are the $\delta^{13}C$ values of the substrate and the product, respectively (Hayes, 1993).

3. Results and discussion

During the 242 days of the experiment CH_4 decreased to 26%, 22%, and 44% of the initial amounts for incubations A, B, and C, respectively, while about 80% of the methane was still present in the control (Fig. 1A). However, the loss of CH_4 in the control was not accompanied by a significant variation in the $\delta^{13}C$ (Fig. 1B).

Rates of the decrease of methane (Table 1) are calculated after subtracting an amount of CH_4 equivalent to the loss observed for the control. The rates calculated for the total incubation time of 242 days ranged between 7.3 and 11.9 µmol per day and per gram dry weight (µmol d^{-1} g_{dw}^{-1}) (Table 1). The corresponding sulphide-production rates (SP) were between 7.5 and 10.7 µmol d^{-1} g_{dw}^{-1} (Table 1). The resulting ratios between CH_4-decrease and sulphide production of 1.4, 1.1, and 0.8 for incubations A, B, and C, respectively, are in accordance with the assumption of a molar ratio of about 1:1 between AOM and sulphate reduction in the Black Sea mats (Michaelis et al., 2002; Nauhaus et al., 2005).

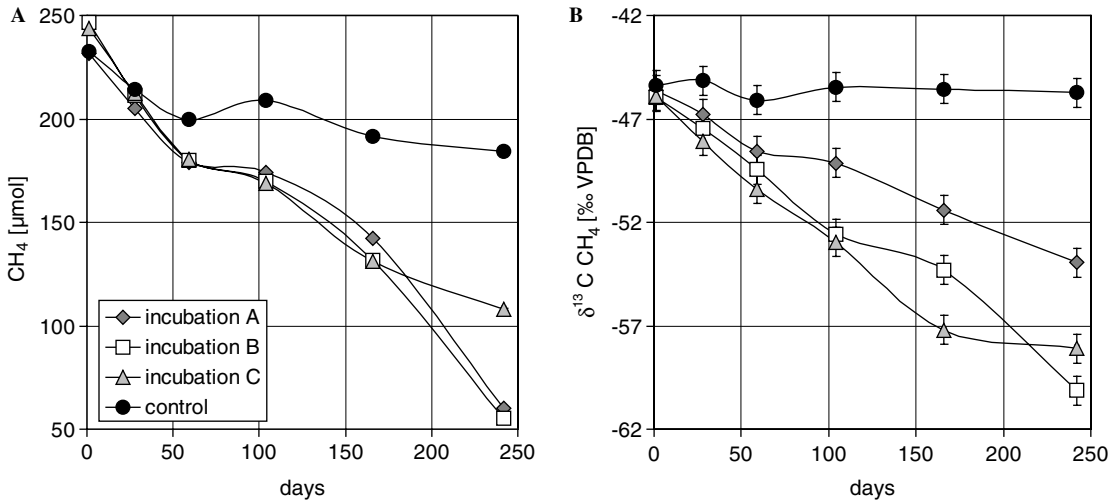

Fig. 1. (A) Changes of CH_4 concentrations (total amounts of CH_4 present in the respective incubation vial) for three parallel incubations of a Black Sea mat (incubations A, B, and C) and a control. (B) Respective changes of signatures of stable carbon isotopes of CH_4.

Table 1
Mean rates of methane-decrease (MD) and sulphide-production (SP) per day and per gram dry weight of microbial mat [µmol d^{-1} g_{dw}^{-1}] for the three incubations depicted in Fig. 1

Days	A		B		C	
	MD	SP	MD	SP	MD	SP
0–28	5.8		12.1		9.4	
28–59	7.4		10.6		10.9	
59–104	6.2		8.8		9.3	
104–166	4.7		6.7		6.5	
166–242	19.8		18.2		4.3	
0–242	10.2	7.5	11.9	10.7	7.3	9.2

Similar anoxic incubations of homogenised microbial mats of the Black Sea yield CH_4-dependent sulphate-reduction rates ranging from 15 to 81 µmol d^{-1} g_{dw}^{-1} with an average of 41± 16 µmol d^{-1} g_{dw}^{-1} (Nauhaus et al., 2005). These incubations were performed at a CH_4 partial pressure of 0.1 MPa (1 atm), twice that initially present during the incubations reported here. The relatively low rates we measured might be attributed to the relatively low CH_4 pressure. Nauhaus et al. (2005) incubated Black Sea mats and Hydrate Ridge sediments performing the AOM at different partial pressures of CH_4. Increasing the CH_4 pressure from 0.1 to 1.1 MPa provoked a fivefold higher sulphate-reduction rate in the Hydrate Ridge sediment but only doubled the rate for the Black Sea mats. However, the CH_4-decrease rates did not become minor with the ongoing decrease of CH_4 during the incubations as would be expected assuming CH_4 pressure to be the controlling parameter (Table 1).

We found a continuous depletion in ^{13}C of the residual CH_4 with decreasing CH_4 concentrations for all three incubations (Fig. 1B). A preferential use of ^{13}C–CH_4 during AOM is extremely unlikely. In fact, all studies on the kinetic carbon isotope fractionation accompanying AOM showed an enrichment of ^{13}C for the residual CH_4 with fractionation factors (αC) in the range of 1.002–1.014 (Whiticar and Faber, 1986; Alperin et al., 1988; Martens et al., 1999; Grossman et al., 2002). Calculations based on CH_4 concentrations and $\delta^{13}C$–CH_4 data of a sediment push-core taken in the Black Sea close to the sampling site of the microbial mats (Treude et al., 2005), lead to an αC for the CH_4 consumption of 1.009. The anaerobic methanotrophic community within this core was similar to the incubated mats dominated by ANME-1 (Treude et al., 2005). Thus, the observed depletions in ^{13}C of the residual CH_4 during the 242 days incubation period (8.3‰, 14.2‰, and 12.2‰ for incubations A, B, and C, respectively) obtained here are the opposite of what is expected if CH_4 concentrations are controlled exclusively by anaerobic methanotrophy. However, Pimenov et al. (1997) found by a radiotracer approach indications for the capability of microbial mats of the Black Sea to perform both, AOM and methanogenesis. Geochemical and phylogenetic investigations of prokaryotic communities in methane seep habitats of the southern Ryukyu Arc also indicated AOM and methanogenesis to occur in close proximity (Inagaki et al., 2004). Hoehler et al. (2000) studied AOM in gas hydrate-

bearing sediments of the Blake Ridge and found most ^{13}C–depleted CH_4, where they suggested high AOM-rates from data on sulphate-reduction rates. Increasing $\delta^{13}C$–CH_4 accompanied by decreasing CH_4 concentrations signatures directly above that zone were attributed to AOM by these authors. We thus assumed that the generation of ^{13}C–depleted CH_4 might mask the experimental kinetic isotope fractionation of the CH_4-oxidation. To test this assumption, we performed three incubations under the same conditions as for the other incubations but substituting CH_4 by N_2. CH_4-production was found for all three incubations with a mean $\delta^{13}C$–CH_4 of −81‰ (−80.4‰, −80.4‰, and −82.3‰). Figure 2B illustrates the effects of CH_4-oxidation, CH_4-production, and both processes taking place concurrently on the concentration and isotopic composition of CH_4 in a closed system. The calculations were done assuming an αC of 1.009 for CH_4-oxidation and a $\delta^{13}C$ of −81‰ for the CH_4 generated. Thus, our data can be fairly well interpreted by assuming both processes having co-occurred in the incubations with a ratio between CH_4-oxidation and CH_4-generation of about 2:1 (Fig. 2A and B). Rates of CH_4-oxidation and CH_4-generation for our incubation experiments calculated between the sampling points and for the total running period of 242 days are shown in Table 2.

For a time period from t_0 to t_1, the amounts of CH_4 generated or oxidised are given by

$$^{12}CH_{4(t_1)} = {}^{12}CH_{4(t_0)} + \left({}^{12}CH_{4(MG)} - {}^{12}CH_{4(MO)}\right)_{t_0-1}$$

$$^{13}CH_{4(t_1)} = {}^{13}CH_{4(t_0)} + \left({}^{13}CH_{4(MG)} - {}^{13}CH_{4(MO)}\right)_{t_0-1}$$

with $CH_{4(t_1)}$ being the amount of CH_4 present at t_1, $CH_{4(t_0)}$ the amount of CH_4 present at t_0, $CH_{4(MG)}$ the amount of CH_4 produced, and $CH_{4(MO)}$ the amount of CH_4 oxidised for $^{12}CH_4$ and $^{13}CH_4$, respectively.

Calculated rates of anaerobic oxidation of methane (AOMR) of microbial mat between sampling points range from 8.5 to 38.1 μmol d^{-1} g_{dw}^{-1}. For the total time of incubation AOMR were 17.5 (incubation A), 24.7 (incubation B), and 17.9 μmol d^{-1} g_{dw}^{-1} (incubation C), while the respective rates of CH_4-production (MPR) were 7.3 (incubation A), 12.9 (incubation B), and 10.6 μmol d^{-1} g_{dw}^{-1} (incubation C). The AOMR/MPR ratios are 2.39 (incubation A), 1.92 (incubation B), and 1.68 (incubation C). Hence, the rates of AOM during the incubations were about twice those found for SR putting a stoichiometry of 1:1 between AOM and SR (Boetius et al., 2000;

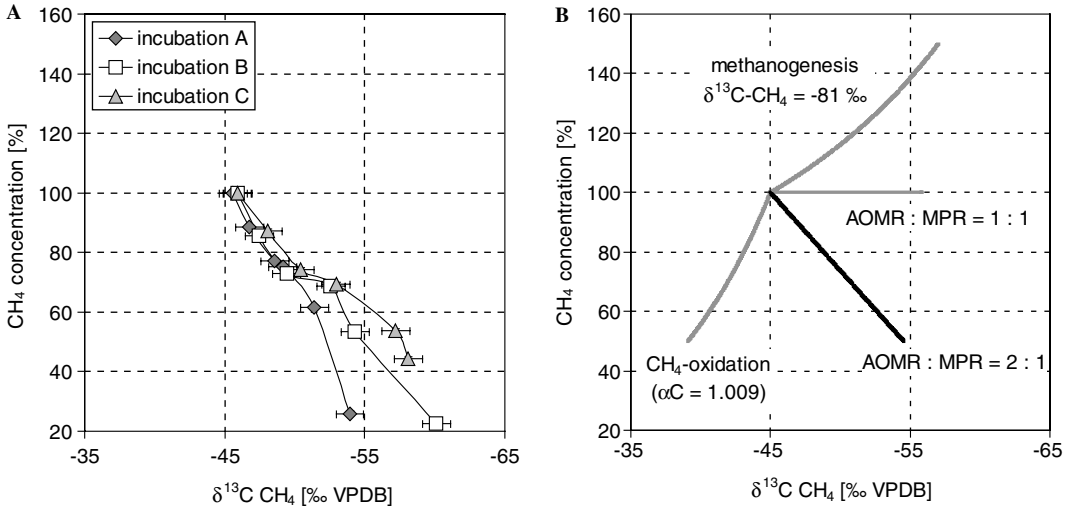

Fig. 2. (A) Measured changes of the concentration and isotopic composition of CH_4 during 242 days of incubation of a Black Sea mat (see also Fig. 1). (B) Modelled shifts of the concentration and isotopic composition of CH_4 in a closed system during CH_4-oxidation, CH_4-production, and both processes taking place concurrently. Initial conditions chosen are a CH_4-concentration of 100% and a $\delta^{13}C$–CH_4 of −45‰ (like the $\delta^{13}C$ of the CH_4 used for the incubation experiments). AOMR = Rate of anaerobic CH_4-oxidation of; MPR = Rate of CH_4-production. Note that the observed variations (A) are very well mirrored by the curve calculated for a concurrent presence of CH_4-oxidation and CH_4-production with a AOMR:MPR of 2:1 (black curve in B).

Table 2
Calculated rates of anaerobic oxidation of methane (AOMR), methane production (MPR) per day and per gram dry weight of microbial mat [μmol d^{-1} g_{dw}^{-1}], and AOMR/MPR ratios for incubations A, B, and C

Days	A			B			C		
	AOMR	MPR	Ratio	AOMR	MPR	Ratio	AOMR	MPR	Ratio
0–28	14.2	8.4	1.69	24.9	12.8	1.95	25.8	16.4	1.58
28–59	18.6	11.2	1.66	24.3	13.6	1.78	26.8	15.9	1.68
59–104	10.4	4.2	2.46	23.8	15.0	1.58	22.9	13.6	1.68
104–166	11.4	6.7	1.70	13.8	7.1	1.94	20.8	14.3	1.46
166–242	31.4	11.6	2.71	38.1	19.9	1.92	8.5	4.2	2.02
0–242	17.5	7.3	2.39	24.7	12.9	1.92	17.9	10.6	1.68

Calculations are done from amounts and $\delta^{13}C$ of methane (Fig. 1) using a kinetic fraction factor αC of 1.009 for the AOM and $\delta^{13}C$ of −81‰ for the methane produced.

Nauhaus et al., 2002; Nauhaus et al., 2005) into question, at least for ANME-1 dominated settings.

Results shown in Table 2 based on an assumed αC of 1.009 for AOM as derived from published estimates (Whiticar and Faber, 1986; Alperin et al., 1988; Martens et al., 1999) and observations in a Black Sea sediment push-core (Treude et al., 2005). However, these calculations were done on field data without taking into account a possible CH_4-production, that, according to our incubation experiments, may have influenced the measured CH_4 concentrations as well as the isotopic signatures. Addition of ^{13}C–depleted CH_4 from methanogenesis would result in an underestimation of the actual fractionation during AOM. Similarly, the $\delta^{13}C$–CH_4 of −81‰ we measured for CH_4-production might be too high as partial oxidation of the released CH_4 is likely. Thus, we also calculated AOMR and MPR assuming a higher fractionation factor of αC of 1.011 and production of more ^{12}C-enriched CH_4 ($\delta^{13}C$–CH_4 of −91‰ and −101‰). Application of a higher fractionation factor leads to lower AOMR/MPR ratios while assuming the production of more ^{12}C–enriched CH_4 results in higher AOMR/MPR ratios (Table 3). However, significant MPR with AOMR/MPR ratios <3.5 result for all considered scenarios.

This result is surprising, as methanogenesis is generally thought to be severely curtailed in sulphate-rich milieus due to outcompeting of methanogenic archaea by sulphate-reducing bacteria for competitive substrates, in the case of carbonate reduction for available hydrogen (Martens and Berner, 1974). Thus, methanogenesis based on non-competitive substrates as methanol or methylamines (Oremland et al., 1982; Oremland and Polcin, 1982) seems likely. To check this possibility, we incubated an active AOM-performing Black Sea mat from the same sampling location as that used for the other experiments in artificial seawater medium adding different substrates (Table 4). CH_4-production rates (MPR) were 7 ± 1.5 μmol d^{-1} g_{dw}^{-1} without additional substrate. The addition of hydrogen, acetate or formate did not lead to a strong stimulation of the MPR over an incubation period of two weeks,

Table 3
Ratios between rates of the anaerobic oxidation of methane (AOMR) and methane production (MPR) per day and per gram dry weight of microbial mat [μmol d^{-1} g_{dw}^{-1}] assuming different fractionation factors (αC) for AOM and different isotopic compositions for the methane produced

αC AOM	$\delta^{13}C$ MP [‰]	A	B	C
		AOMR/MPR	AOMR/MPR	AOMR/MPR
1.009	−81	2.39	1.92	1.68
1.009	−91	2.89	2.46	2.04
1.009	−101	3.49	2.98	2.39
1.011	−81	2.15	1.74	1.57
1.011	−91	2.71	2.24	1.91
1.011	−101	3.27	2.72	2.24

Calculations are done for the total time of incubation (0–242 days) for all incubation (A, B, and C).

Table 4
Rates of methane production (MPR) per day and per gram dry weight of a Black Sea microbial mat [μmol d^{-1} g_{dw}^{-1}] in the presence of sulphate (artificial seawater medium with 16 mM sulphate) without (control) and with different substrates added

Substrate	Substrate concentration at beginning	MPR	SE
Control		6.95	1.45
H_2	80%	9.44	2.27
Acetate	10 mM	7.74	2.01
Formate	10 mM	8.01	2.05
TMA	10 mM	14.25	2.75
Methanol	5 mM	12.11	1.07

SE = standard error of three parallels; TMA = trimethylamine.

most likely a consequence of the intensive competition for these substances of methanogenic archaea with sulphate-reducing bacteria. However, following the addition of the non-competitive substrates methanol or trimethylamine MPR significantly increased to 12 ± 1 and 14 ± 3 μmol d^{-1} g_{dw}^{-1}, respectively. Generally, these MPR in the presence of sulphate meet those required to account for the analytical data of incubations A, B, and C (Tables 2 and 3).

Within our experiments, the difference between the isotopic composition of the CH_4 consumed ($\delta^{13}C = -45‰$ for the CH_4 added to the system) and the CH_4 generated ($\delta^{13}C = -81‰$) was about 36‰ while *in situ* this difference is generally very small. Therefore, an isotopic shift with ^{13}C depletion in the residual CH_4 as observed in our incubations is not to be expected for *in situ* studies. In fact, assuming that the CH_4 consumed has the same isotopic signature as the methane generated, the residual CH_4 would become ^{13}C enriched even if the rates of methanogenesis are only slightly below those of the AOM. However, disregarding an existing methanogenesis would generally lead to an underestimation of the isotopic fraction of the AOM, as long as the CH_4 generated is not more ^{13}C enriched compared to the CH_4 pool.

4. Conclusions

The methane turnover of a microbial mat performing the anaerobic oxidation of methane could be traced in incubation experiments. The results revealed new insights into this process of high relevance for the methane budgets of marine sediments. Notably, anaerobic methanotrophy and microbial methanogenesis can take place simultaneously in sulphate-rich environments. Moreover, our data show that the rates of methane production and consumption can be in the same order of magnitude. Several aspects derived from our study are highly relevant for investigations of the methane dynamics in anoxic settings.

1. Records of methane concentrations alone do not allow for a calculation of exact methane oxidation rates.
2. The molar ratio between anaerobic methanotrophy and sulphate reduction is not necessarily 1:1, but might be considerably higher. Therefore, the anaerobic oxidation of methane might be understated by estimations based on sulphate-reduction or sulphide-production rates.
3. Methane production takes place in the presence of sulphate, most probably based on substrates non-competitive to sulphate-reducing bacteria like methylamines and methanol.
4. For estimations of AOM rates and of the accompanying kinetic carbon isotope fractionation from field data and laboratory experiments the possibility of a parallel occurrence of anaerobic methanotrophy and methanogenesis should be considered. An underestimation of fractionation factors (αC) for anaerobic methanotrophy may be caused by unrecognised methanogenesis within the systems investigated.

Acknowledgements

We thank the crews of the R.V. 'PROFESSOR LOGACHEV' and of the research submersible 'JAGO' for excellent collaboration during the field work. This study received financial support through programs GHOSTDABS (03G0559A), MUMM (03G0554A), and METRO (03G0604A) of the Bundesministerium für Bildung und Forschung (BMBF), the Universität Hamburg, and the Max-Planck-Gesellschaft (Germany). This is Publication No. GEOTECH-224 of the GEOTECHNOLOGIEN program of the BMBF and the DFG, and Publication No. 17 of the research program GHOSTDABS.

References

Aeckersberg, F., Bak, F., Widdel, F., 1991. Anaerobic oxidation of saturated hydrocarbons to CO_2 by a new type of sulfate-reducing bacterium. Archives of Microbiology (Historical Archive) 156, 5–14.

Alperin, M.J., Reeburgh, W.S., Whiticar, M.J., 1988. Carbon and hydrogen isotope fractionation resulting from anaerobic methane oxidation. Global Biogeochemical Cycles 2, 279–288.

Barker, J.F., Fritz, P., 1981. Carbon isotope fractionation during microbial methane oxidation. Nature 293, 289–291.

Barnes, R.O., Goldberg, E.D., 1976. Methane production and consumption in anaerobic marine sediments. Geology 4, 297–300.

Beerling, D.J., Berner, R.A., 2002. Biogeochemical constraints on the Triassic–Jurassic boundary carbon cycle event. Global Biogeochemical Cycles 16, 101–1013.

Bergamaschi, P., Harris, G.W., 1995. Measurement of stable isotope ratios ($^{13}CH_4/^{12}CH_4$; $^{12}CH_3D/^{12}CH_4$) in landfill methane using a tunable diode laser absorption spectrometer. Global Biogeochemical Cycles 9, 439–447.

Blumenberg, M., Seifert, R., Nauhaus, K., Pape, T., Michaelis, W., 2005. *In vitro* study of lipid biosynthesis in an anaerobically methane oxidizing microbial mat. Applied and Environmental Microbiology 71, 4345–4351.

Blumenberg, M., Seifert, R., Reitner, J., Pape, T., Michaelis, W., 2004. Membrane lipid patterns typify distinct anaerobic methanotrophic consortia. Proceedings of the National Academy of Sciences of the United States of America 101, 11111–11116.

Boetius, A., Ravenschlag, K., Schubert, C.J., Rickert, D., Widdel, F., Gieseke, A., Amann, R., Jørgensen, B.B., Witte, U., Pfannkuche, O., 2000. A marine microbial consortium apparently mediating anaerobic oxidation of methane. Nature 407, 623–626.

Coleman, D.D., Risatti, J.B., Schoell, M., 1981. Fractionation of carbon and hydrogen isotopes by methane-oxidizing bacteria. Geochimica et Cosmochimica Acta 45, 1033–1037.

de Wit, M.J., Ghosh, J.G., de Villiers, S., Rakotosolofo, N., Alexander, J., Tripathi, A., Cindy, L., 2002. Multiple organic carbon isotope reversals across the Permo-Triassic Boundary of terrestrial Gondwana Sequences: clues to extinction patterns and delayed ecosystem recovery. The Journal of Geology 110, 227–240.

Dickens, G.R., O'Neil, J.R., Rea, D.K., Owen, R.M., 1995. Dissociation of oceanic methane hydrate as a cause of the carbon isotope excursion at the end of the Paleocene. Paleoceanography 10, 965–972.

Grossman, E.L., Cifuentes, L.A., Cozzarelli, I.M., 2002. Anaerobic methane oxidation in a landfill-leachate plume. Environmental Science and Technology 63, 2436–2442.

Hallam, S.J., Putnam, N., Preston, C.M., Detter, J.C., Rokhsar, D., Richardson, P.M., DeLong, E.F., 2004. Reverse methanogenesis: testing the hypothesis with environmental genomics. Science 305, 1457–1462.

Haq, B.U., 1998. Gas hydrates: greenhouse nightmare? Energy panacea or pipe dream? GSA Today 8, 1–6.

Hayes, J.M., 1993. Factors controlling ^{13}C contents of sedimentary organic compounds: principles and evidence. Marine Geology 113, 111–125.

Hoehler, T.M., Borowski, W.S., Alperin, M.J., Rodriguez, N.M., Paull, C.K., 2000. Model, stable isotope, and radiotracer characterization of anaerobic methane oxidation in gas hydrate-bearing sediments of the Blake Ridge. In: Paull, C.K., Matsumoto, R., Wallace, P.J., Dillon, W.P. (Eds.), Proceedings of the Ocean Drilling Program, Scientific Results 164, Ocean Drilling Program, College Station, TX, pp. 79–85.

Inagaki, F., Tsunogai, U., Suzuki, M., Kosaka, A., Machiyama, H., Takai, K., Nunoura, T., Nealson, K.H., Horikoshi, K., 2004. Characterization of C1-metabolizing prokaryotic communities in methane seep habitats at the Kuroshima Knoll, Southern Ryukyu Arc, by analyzing pmoA, mmoX, mxaF, mcrA, and 16S rRNA genes. Applied and Environmental Microbiology 70, 7445–7455.

Krüger, M., Meyerdierks, A., Glöckner, F.O., Amann, R., Widdel, F., Kube, M., Reinhardt, R., Kahnt, J., Böcher, R., Thauer, R.K., Shima, S., 2003. A conspicuous nickel protein in microbial mats that oxidize methane anaerobically. Nature 426, 878–881.

Krüger, M., Treude, T., Wolters, H., Nauhaus, K., Boetius, A., 2005. Microbial methane turnover in different marine habitats. Palaeogeography, Palaeoclimatology, Palaeoecology 227, 6–17.

Kvenvolden, K.A., Ginsburg, G.D., Soloviev, V.A., 1993. Worldwide distribution of subaquatic gas hydrates. Geo-Marine Letters 13, 32–40.

Kvenvolden, K.A., Lorenson, T.D., 2001. The global occurrence of natural gas hydrates. In: Paull, C.K., Dillon, W.P. (Eds.), Natural Gas Hydrates: Occurrence, Distribution, and Detection, Geophysical Monograph Series, vol. 124. American Geophysical Union, Washington, DC, pp. 3–18.

Martens, C.S., Albert, D.B., Alperin, M.J., 1999. Stable isotope tracing of anaerobic methane oxidation in the gassy sediments of Eckernförde Bay, German Baltic Sea. American Journal of Science 299, 589–610.

Martens, C.S., Berner, R.A., 1974. Methane production in the interstitial waters of sulfate-depleted marine sediments. Science 185, 1167–1169.

Michaelis, W., Seifert, R., Nauhaus, K., Treude, T., Thiel, V., Blumenberg, M., Knittel, K., Gieseke, A., Peterknecht, K., Pape, T., Boetius, A., Amann, R., Jørgensen, B.B., Widdel, F., Peckmann, J., Pimenov, N.V., Gulin, M.B., 2002. Microbial reefs in the Black Sea fueled by anaerobic oxidation of methane. Science 297, 1013–1015.

Nauhaus, K., Boetius, A., Krüger, M., Widdel, F., 2002. *In vitro* demonstration of anaerobic oxidation of methane coupled to sulphate reduction in sediment from a marine gas hydrate area. Environmental Microbiology 4, 296–305.

Nauhaus, K., Treude, T., Boetius, A., Krüger, M., 2005. Environmental regulation of the anaerobic oxidation of methane: comparison of ANME-I and ANME-II-communities. Environmental Microbiology 7, 98–106.

Orcutt, B., Boetius, A., Elvert, M., Samarkin, V., Joye, S.B., 2005. Molecular biogeochemistry of sulfate reduction, methanogenesis and the anaerobic oxidation of methane at Gulf of Mexico cold seeps. Geochimica et Cosmochimica Acta 69, 4267–4281.

Oremland, R.S., Marsh, L.M., Polcin, S., 1982. Methane production and simultaneous sulphate reduction in anoxic, salt marsh sediments. Nature 296, 143–145.

Oremland, R.S., Polcin, S., 1982. Methanogenesis and sulfate reduction: competitive and non competitive substrates in estuarine sediments. Applied and Environmental Microbiology 44, 1270–1276.

Petit, J.R., Jouzel, J., Raynaud, D., Barkov, N.I., Barnola, J.-M., Basile, I., Bender, M., Chappellaz, J., Davis, M., Delaygue, G., Delmotte, M., Kotlyakov, V.M., Legrand, M., Lipenkov, V.Y., Lorius, C., Pepin, L., Ritz, C., Saltzman, E., Stievenard, M., 1999. Climate and atmospheric history of the past 420,000 years from the Vostok ice core, Antarctica. Nature 399, 429–436.

Pimenov, N.V., Rvisanov, I.I., Poglazova, M.N., Mityushina, L.L., Sorokin, D.Y., Khmelenina, V.N., Trotsenko, Y.A., 1997. Bacterial mats on an coral-like structures at methane seeps in the Block Sea. Mikrobiologiya 66, 354–360.

Reeburgh, W.S., 1976. Methane consumption in Cariaco Trench waters and sediments. Earth and Planetary Science Letters 28, 337–344.

Reeburgh, W.S., Hirsch, A.I., Sansone, F.J., Popp, B.N., Rust, T.M., 1997. Carbon kinetic isotope effect accompanying microbial oxidation of methane in boreal forest soils. Geochimica et Cosmochimica Acta 61, 4761–4767.

Silverman, M.P., Oyama, V.I., 1968. Automatic apparatus for sampling and preparing gases for mass spectral studies of carbon isotope fractionation during methane metabolism. Analytical Chemistry 40, 1833–1877.

Treude, T., Knittel, K., Blumenberg, M., Seifert, R., Boetius, A., 2005. Subsurface microbial methanotrophic mats in the Black

Sea. Applied and Environmental Microbiology 71, 6375–6378.

Tyler, S.C., Crill, P.M., Brailsford, G.W., 1994. $^{13}C/^{12}C$ fractionation of methane during oxidation in a temperate forested soil. Geochimica et Cosmochimica Acta 58, 1625–1633.

Whiticar, M.J., Faber, E., 1986. Methane oxidation in sediment and water column environments – isotope evidence. Organic Geochemistry 10, 759–768.

Wuebbles, D.J., Hayhoe, K., 2002. Atmospheric methane and global change. Earth-Science Reviews 57, 177–210.