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Characterization of methanogenic Archaea and stable isotope fractionation during methane production in the profundal sediment of an oligotrophic lake (Lake Stechlin, Germany)

Ralf Conrad
Max-Planck-Institute for Terrestrial Microbiology, Karl-von-Frisch-Str., 35043 Marburg, Germany

On-Chim Chan
Max-Planck-Institute for Terrestrial Microbiology, Karl-von-Frisch-Str., 35043 Marburg, Germany; Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, 650223 Kunming, China; Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Department of Limnology of Stratified Lakes, 16775 Stechlin, Germany

Peter Claus
Max-Planck-Institute for Terrestrial Microbiology, Karl-von-Frisch-Str., 35043 Marburg, Germany

Peter Casper
Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Department of Limnology of Stratified Lakes, 16775 Stechlin, Germany

Abstract

The methanogenic archaeal community, methane production, and carbon isotopic fractionation were studied in the profundal sediments of oligotrophic Lake Stechlin. Because the water column of oligotrophic lakes is usually oxic, CH_4 is normally not produced in the sediment surface layers. Nevertheless, 16S rRNA gene sequences of both acetoclastic *Methanosaeta* spp. and hydrogenotrophic *Methanomicrobiales* were detected, and potential CH_4 production (assayed at 30°C) was much higher at the surface (0–5 cm) than in the deeper (20–25 cm) sediment layers, albeit starting only after sulfate was reduced. Copy numbers of the bacterial 16S rRNA genes were also higher in the surface than in the deeper sediment layers, but those of archaeal 16S rRNA genes were similar. Hydrogenotrophic and acetoclastic methanogenesis contributed equally to methane production, as quantified by a comprehensive analysis of $\Delta^{13}C$ in organic carbon, acetate, CO_2, and CH_4 and by determination of the isotopic enrichment factor for conversion of CO_2 to CH_4. This value ($\approx -78‰$) indicates that the Gibbs free energy of hydrogenotrophic methanogenesis was more negative, implying the energetic conditions in situ were more favorable, than suggested by measured H_2 partial pressures. The fermentatively produced acetate-methyl was by about 10‰ lighter than the $\Delta^{13}C$ of sediment organic carbon, indicating an unusual fractionation during fermentative production of acetate. Homoacetogenesis from CO_2 probably played only a minor role because the intramolecular difference between the $\Delta^{13}C$ of the carboxyl and the methyl groups of acetate was only on the order of 20‰. A remarkable microbial community is able to produce methane from organic matter in the oxidized surface layer of oligotrophic lake sediments in which methane normally is not produced, so that the ecological relevance of this potential is presently unclear.

In sediments of freshwater lakes, methanogenic degradation of organic matter is the main path of carbon mineralization (Rudd and Taylor 1980). However, in mesotrophic or oligotrophic lakes, in which the hypolimnion stays oxic year round, production of methane is usually suppressed at the surface and only occurs in the deeper sediment layers (Lovley and Klug 1983; Kuivila et al. 1989). In contrast to CH_4 production in deep layers, little is known about the potential CH_4 production in the surface layers of sediments, which are mostly oxidized. In fact, these layers are usually dominated by sulfate reduction, and methanogenesis is not expected (Lovley and Klug 1983; Kuivila et al. 1989).

Methane is produced by a complex microbial community that degrades organic matter under anaerobic conditions to CH_4 and CO_2. The production of CH_4 thereby is accomplished by methanogenic Archaea, which use either acetate or $H_2 + CO_2$ as substrates, which in turn are produced by fermentation of organic matter (Conrad 1999). However, comparatively little is known about the composition of the community of methanogenic Archaea in lake sediments (Zepp-Falz et al. 1999; Chan et al. 2002; Koizumi et al. 2003). Recently, we have shown that both the major path of CH_4 production and the composition of the methanogenic archaeal community changes with depth of the profundal sediment of a eutrophic lake, which

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develops an anoxic hypolimnion during summer stratification (Chan et al. 2005). The sediment of this eutrophic lake is accumulating, with 10 cm depth representing about 40 years of sedimentation. The sedimentation in oligotrophic lakes is slower. Furthermore, in such lakes, the top sediment layers will be dominated by sulfate reduction rather than methanogenesis.

We were interested in whether the methanogenic community in oligotrophic lake sediment changes with depth, in particular whether a methanogenic community also occurs in the upper sediment layers and whether it is able to express methanogenic activity. Therefore, we measured the CH_4 production potential by incubation at 30°C. We chose an elevated incubation temperature because we were only interested in the potential rather than the in situ activity and because CH_4 production rates at close to in situ temperature were very low and required long incubation times for measurement. In addition, the biogeochemistry of potential methane production in these layers was investigated by stable isotope analysis in the sediment incubations.

Little is known about stable carbon isotope fractionation during methanogenesis in lake sediments. Although methanogenesis from $H_2 + CO_2$ strongly prefers the isotopically lighter carbon, the isotope effect seems to be expressed less during methanogenesis from acetate (Whiticar et al. 1986; Hornibrook et al. 2000; Conrad 2005). This difference in isotopic fractionation can in principle be used to calculate the relative contribution of the two methanogenic pathways to total CH_4 production when the following stable isotopic composition and isotopic fractionation factors are known for the environmental system (Conrad 2005): $\delta^{13}C$ of CH_4 (δ_{CH4}), CO_2 (δ_{CO2}), and acetate-methyl ($\delta_{ac\text{-}methyl}$), as well as the fractionation factors for the reduction of CO_2 to CH_4 (α_{mc}) and acetate-methyl to CH_4 (α_{ma}). Alternatively, the isotopic enrichment factor ($\varepsilon = [1 - \alpha]10^3$) can be used. Isotopic values of δ_{CH4} and δ_{CO2} have occasionally been investigated in lake sediments (Whiticar et al. 1986; Hornibrook et al. 2000; Conrad 2005), but only few studies have determined $\delta_{ac\text{-}methyl}$ and estimated fractionation factors (Gelwicks et al. 1994; Penning et al. 2006*a*).

Because virtually nothing is known about the mechanisms and biogeochemistry of methanogenesis in the surface sediment of oligotrophic lakes, we studied the methane production potential, stable isotope biogeochemistry, and methanogenic archaeal community in the surface layers and also in deeper sediment layers of Lake Stechlin, an oligotrophic lake in northern Brandenburg, Germany. The basic aspects of methane cycling (Casper et al. 2003) and sulfate reduction (Sass et al. 2003) have already been studied in this lake.

Materials and methods

Sampling procedure—Oligotrophic Lake Stechlin is located in northern Brandenburg, Germany. The main morphological and limnological characteristics of the lake were described by Casper (1985), and results of recent studies were summarized in Koschel and Adams (2003). The temperature and dissolved oxygen in the water column (32 m deep at station Stechlin south) were measured with an Oxi 197 probe (WTW) immediately before taking sediment samples. Sediment cores (diameter 7.5 cm) were sampled with a Jenkin sediment sampler on 10 September 2003 (for clone library) and on 04 November 2004 (for the other analyses). Triplicate cores were processed within 1 h. The sediment cores were sliced into subsections under a flow of N_2. Aliquots for quantification of 16S rRNA gene copies were pooled and stored frozen (-80°C). Pore-water concentrations of nitrate and sulfate were analyzed by ion chromatography (DX-100; Dionex) after centrifugation of aliquots of the triplicate core sections and membrane filtration of the supernatants. Values of pH were measured with a pH meter (pH3000, WTW). Methane concentrations in the pore water were determined by gas chromatography after extraction of aliquots of the core sections as described by Casper et al. (2003). The sections from 0 to 5 and 20 to 25 cm depth were stored for each core separately in serum bottles at 4°C and used for incubation studies within 1 week.

Incubation experiments—For sediment incubations, 25-mL aliquots (about 1.4 g dry weight) of each triplicate sediment sampled on 04 November 2004 were transferred into 120-mL sterile serum bottles, flushed with N_2, closed with butyl rubber stoppers, and incubated at 4°C overnight. The exact amount of sediment was determined gravimetrically. Then, the bottles were flushed again with N_2 and further incubated at 30°C. The gas headspace of some of the bottles was supplemented with 2% CH_3F (Fluorochrome). Gas samples (200 μL for each of three analyses) were repeatedly taken from the headspace of the bottles with a gas-tight pressure lock syringe (Dynatech) after the bottles were vigorously shaken by hand and analyzed immediately for (1) CH_4 and CO_2, (2) H_2, and (3) $\delta^{13}C$ of CH_4 and CO_2. At the end of incubation, the bottles were sacrificed for sampling of the liquid phase. Aliquots of the slurry were centrifuged, the supernatants were filtered through 0.2-μm polytetrafluoroethylene (PTFE) membrane filters (REZIST 13-0.2 PTFE, Schleicher & Schüll) and stored frozen (-20°C) for later analysis of concentration and $\delta^{13}C$ of acetate. The dry weight of the sample was determined gravimetrically. The $\delta^{13}C$ of sediment carbon (δ_{tot}) was analyzed after air drying of sediment aliquots at room temperature. The $\delta^{13}C$ of particulate organic carbon (δ_{org}) was measured after removal of carbonate carbon (δ_{carb}) by addition of HCl until bubble formation of CO_2 had ceased, followed by air drying of the sediment slurry at room temperature. The value of δ_{carb} was calculated from δ_{tot} and δ_{org} by difference.

Quantification of 16S rRNA gene fragments—The DNA of the sediment sample was extracted with the Soil DNA Isolation Kit (MO BIO Laboratories) following the manufacturer's instructions, as described in detail by Chan et al. (2005). The abundance of bacterial and archaeal 16S rRNA genes was determined in sediment sampled on 04 November 2004 by "real-time" polymerase chain reaction (PCR) assays on the basis of detection by SYBR green fluorescence with the SYBR Green Jumpstart

TaqReadyMix (Sigma). An ABI Prism 7000 Sequence Detection System (Applied Biosystems) was used for PCR. The extracted DNA was diluted to 1 : 2, 1 : 5, and 1 : 10 with double distilled water. Each PCR reaction mixture contained 25 μL of SYBR® Green Master Mix (Applied Biosystems), 1.5 μL (10 μmol L^{-1}) of each the forward and the reverse primers, 0.5 μL (20 mg mL^{-1}) bovine serum albumin (Roche Diagnostics), 2 μL of diluted DNA sample, and water to a total volume of 50 μL. The domain-specific primer sets Ba519f-Ba907r (Stubner 2002) and Ar109f-Ar915r (Grosskopf et al. 1998*a*) were used for quantification of Bacteria and Archaea, respectively. Each run included three blank samples, which did not contain sample DNA, and a duplicate of a serial 10-fold dilution of standard (10^8–10^1). Almost full-length rRNA gene amplicons of *Escherichia coli* and *Methanosarcina barkeri* were used as standards for Bacteria and Archaea quantification, respectively (Lueders et al. 2004). The thermal profile used was: 10 min at 96°C, followed by 40 cycles of 30 s at 96°C, 30 s at 52°C, and 1 min at 72°C for Bacteria or 1.5 min for Archaea quantification, then a final extension at 72°C for 5 min. Dissociation curves were obtained at the end of each run to ensure specificity of the PCR amplicons produced. All samples that showed unspecific dissociation properties were rejected. The threshold number of cycles was determined as the maximum increase of the fluorescent signal for the samples in each run.

Cloning, screening, sequencing, and phylogenetic analysis—Clone libraries of archaeal 16S rRNA gene fragments were constructed with the use of DNA retrieved from sediment sampled on 10 September 2003. Archaeal 16S rRNA gene fragments were amplified with the primer combination Ar109f-Ar915r (Grosskopf et al. 1998*a*), and the PCR products were purified with the QIAquick Gel Extraction Kit (QIAGEN). The purified amplicons were cloned with the TOPO TA Cloning® Kit for Sequencing (Invitrogen) following the manufacturer's instruction.

More than 100 clone colonies were picked randomly for each library and were screened by denaturant gradient gel electrophoresis (DGGE) with the use of the primers 519f-GC and Ar915r for amplification (Oevreas et al. 1997) and the following thermal profile: denaturation at 94°C for 3 min; 25 cycles at 94°C for 30 s, 52°C for 30 s, and 72°C for 30 s; and a final elongation at 72°C for 5 min. The PCR products were analyzed with a DGene System (BioRad). The denaturant gradient was 30–50% of a solution consisting of 7 mol L^{-1} urea and 40% deionized formamide. Electrophoresis was performed at 60°C, 80 V for 15 min, followed by 200 V for 5 h. The gel was then stained with SYBR green I (Biozym) and photographed by AlphaImager 2200 (Alpha Innotech Corp.). Clones with a characteristic DGGE banding position were considered representing an operational taxonomic unit (OTU).

At least one representative clone from each OTU was sequenced. The nucleotide sequences of the cloned 16S rRNA gene fragments were determined with a 3100 Avant Genetic Analyzer (Applied Biosystem) and the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystem). The obtained sequences were checked with the BLAST search program at the National Center for Biotechnology Information (NCBI) web site (www.ncbi.nlm.nih.gov). Sequences were manually aligned to the closest relative provided by NCBI with the Multicolor Sequence Alignment Editor from Hepperle (http://scienc.do-mix.de/aligh.php). Sequences were submitted to the CHECK_CHIMERA program in Ribosomal Database Project (http://www.wdcm.nig.ac.jp/RDP/cgis/chimera.cgi?su=SSU) to exclude chimeric artifacts. Phylogenetic analyses were restricted to nucleotide positions that could be aligned unambiguously in all sequences. Phylogenetic trees were constructed by maximum likelihood, parsimony and neighbor-joining algorithms provided by the ARB program (Ludwig et al. 2004) and PHYLIP (Felsenstein 2004). Nodes of the tree were tested by 100-fold bootstrap analysis.

The clone sequences generated in this study were deposited in the GenBank database under the accession numbers AY531721–AY531752.

Chemical analyses—Methane and CO_2 were analyzed by gas chromatography with a flame ionization detector (GC-8A; Shimadzu) after separation at 40°C on Poropack QS 50-100 mesh (2 m length, 3.2 mm diameter), with H_2 at 30 mL min^{-1} as carrier gas. Carbon dioxide was detected after conversion to CH_4 with a methanizer (NiCr-Ni-catalyst at 350°C; 20 cm length, 3.2 mm diameter; Chrompack). Hydrogen was analyzed by gas chromatography with a HgO-to-Hg conversion detector (280°C, RGD2; Trace Analytical) after separation at 85°C on Molecular Sieve 5 Å (2 m length, 3.2 mm diameter), with synthetic air (80% N_2, 20% O_2) at 10 mL min^{-1} as carrier gas. Gas chromatographs were calibrated with standard gases (Messer Griesheim). Acetate was analyzed on the high-performance liquid chromatography–liquid chromatography–isotope ratio mass spectrometer (HPLC-LC-IRMS) system described below, having a detection limit of about 5 μmol L^{-1}. The HPLC was calibrated with aqueous standard mixtures of acetate and other fatty acids.

Stable carbon isotope analysis in gas samples was performed with a gas chromatograph combustion IRMS (GC-C-IRMS) system (Thermoquest). Its operation is described by Brand (1996). The CH_4 and CO_2 in the gas samples (200 μL) were first separated in a Hewlett Packard 6890 gas chromatograph with a Pora Plot Q column (27.5 m length, 0.32 mm interior diameter; 10 μm film thickness; Chrompack) at 30°C with He (99.996% purity; 2.6 mL min^{-1}) as carrier gas. After conversion of CH_4 to CO_2 in the Finnigan Standard GC Combustion Interface III, the $^{13}C : ^{12}C$ was analyzed by the IRMS instrument (Finnigan MAT model delta plus). The isotope reference gas was CO_2 (99.998% purity; Messer-Griessheim) calibrated with the working standard methylstearate (Merck). The latter was intercalibrated at the Max-Planck-Institut für Biogeochemie, Jena, Germany (courtesy of W. A. Brand), against NBS 22 and USGS 24 and reported in the delta notation versus V-PDB: $\delta^{13}C = 10^3(R_{sa}/R_{st} - 1)$ with $R = {}^{13}C : {}^{12}C$ of sample (sa) and standard (st), respectively. The precision of repeated analysis was ±0.2‰ when 1.3 nmol of CH_4 was injected.

Isotopic measurements of acetate were performed on a HPLC-LC-IRMS system (Spectra System P1000, Thermo Finnigan; Mistral, Spark). The HPLC had an injection loop of 50 μL, the separation was at 35°C on an ion exclusion column (Aminex HPX-87-H, Biorad) with 1 mmol L^{-1} of H_2SO_4 at 0.3 mL min^{-1} as eluent, and coupled to Finnigan LC IsoLink (Thermo Electron Corporation) for oxidation of the separated compounds to CO_2 at 99.9°C with 0.42 mol L^{-1} sodium peroxodisulfate and 1.35 mol L^{-1} phosphoric acid as described by Krummen et al. (2004). Isotope ratios were detected on an IRMS (Finnigan MAT delta plus advantage). Isotope reference gas was CO_2 calibrated as described above. The precision of repeated analysis was ±0.3‰ when 3.2 nmol benzoic acid was injected. The analysis resulted in determination of $\delta^{13}C$ of total acetate.

An off-line pyrolysis was conducted to determine $\delta^{13}C$ of the methyl group of acetate ($\delta_{ac\text{-}methyl}$). Acetate in the liquid sample was purified with HPLC by collecting the acetate fraction from each run. The purified sample was added to a strong NaOH solution and dried in a Pyrex tube under vacuum. The dried reactants were pyrolyzed under vacuum at 400°C, converting the carboxyl carbon to CO_2 and the methyl carbon to CH_4 (Blair et al. 1985; Penning et al. 2006*b*). Gas samples were taken, then the $\delta^{13}C$ of the produced CH_4 was analyzed by GC-C-IRMS as above, which is identical to the $\delta^{13}C$ of the methyl carbon. The $\delta^{13}C$ of the carboxyl carbon was calculated from total acetate and methyl-acetate by difference.

Analysis of the ^{13}C in organic matter was carried out at the Institute for Soil Science and Forest Nutrition (IBW) at the University of Göttingen, Germany (courtesy of Heinz Flessa), with an elemental analyzer (Fisons EA 1108) coupled to an IRMS.

Calculations—Fractionation factors for a reaction A → B are defined after Hayes (1993),

$$\alpha_{A-B} = (\delta_A + 1{,}000)/(\delta_B + 1{,}000) \quad (1)$$

sometimes expressed as isotopic enrichment factor $\varepsilon \equiv 10^3(1-\alpha)$. The isotopic signature for a newly formed CH_4 (δ_n) was calculated from the isotopic signatures at two time points $t = 1$ (δ_1) and $t = 2$ (δ_2) by the mass balance equation in Eq. 2,

$$\delta_2 = f_n\delta_n + (1-f_n)\delta_1 \quad (2)$$

with f_n being the fraction of the newly formed C-compound relative to the total at $t = 2$.

The apparent fractionation factor for conversion of CO_2 to CH_4 is given by Eq. 3.

$$\alpha_{app} = (\delta_{CO2} + 1{,}000)/(\delta_{CH4} + 1{,}000) \quad (3)$$

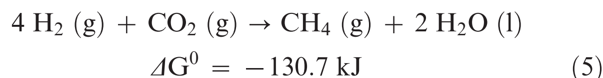
Relative contribution of $H_2 + CO_2$-derived CH_4 to total CH_4 was determined by mass balance Eq. 4 (Conrad 2005),

$$f_{mc} = (\delta_{CH4} - \delta_{ma})/(\delta_{mc} - \delta_{ma}) \quad (4)$$

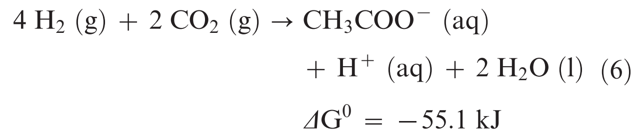
where f_{mc} is the fraction of CH_4 formed from $H_2 + CO_2$, δ_{CH4} is the $\delta^{13}C$ of total produced CH_4, and δ_{ma} and δ_{mc} are the isotope ratios of CH_4 derived from either acetate or $H_2 + CO_2$.

In general, calculations were done with the use of data measured in individual incubation vessels, followed by averaging over the triplicate incubations equivalent to triplicate sediment cores so that the reported data are means ± standard error of triplicates. For data aggregation in tables, mean values of the last five data points at the end of incubation were averaged and are given as mean ± standard error. Total amounts of gases in the headspace of the incubation vessels were calculated from the partial pressures with the volume of the gas space and the gas constant. The amounts dissolved in the liquid were <3% of the total and were neglected.

Gibbs free energies (ΔG) of production of CH_4 or acetate from $H_2 + CO_2$ were calculated from the actual concentrations of reactants and products and the standard Gibbs free energies corrected for the incubation temperature (Conrad and Wetter 1990) with reaction Eqs. 5 and 6.



$$4\,H_2\,(g) + CO_2\,(g) \rightarrow CH_4\,(g) + 2\,H_2O\,(l) \qquad \Delta G^0 = -130.7\ kJ \quad (5)$$



$$4\,H_2\,(g) + 2\,CO_2\,(g) \rightarrow CH_3COO^-\,(aq) + H^+\,(aq) + 2\,H_2O\,(l) \quad (6) \qquad \Delta G^0 = -55.1\ kJ$$

Values of ΔG^0 of the reaction were calculated from tabulated values of the standard Gibbs free energies of formation at 298 K with the reactants and products in the gaseous (g), liquid (l), or aqueous (aq) state as indicated.

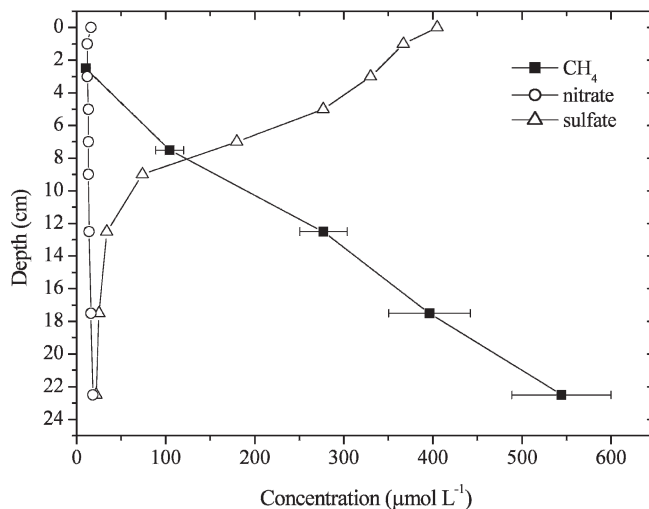


Fig. 1. Vertical profiles of CH_4, nitrate, and sulfate in the profundal sediment of Lake Stechlin, determined on 09 November 2004; mean ± SE, $n = 3$.

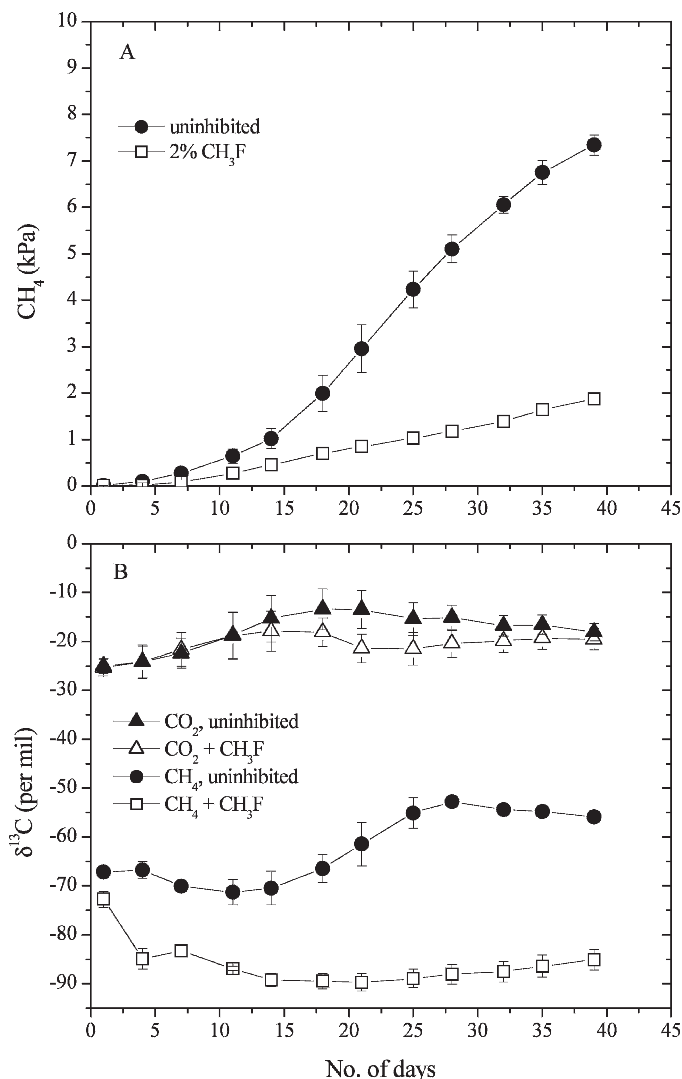


Fig. 2. Potential methane production at 30°C in the surface layers (0–5 cm depth) of profundal sediment of Lake Stechlin in the absence (closed symbols) and presence (open symbols) of methyl fluoride; (A) accumulation of CH_4; (B) $\delta^{13}C$ of the accumulated CH_4 and CO_2; mean ± SE, n = 3; no error bars are shown if smaller than the size of the symbol.

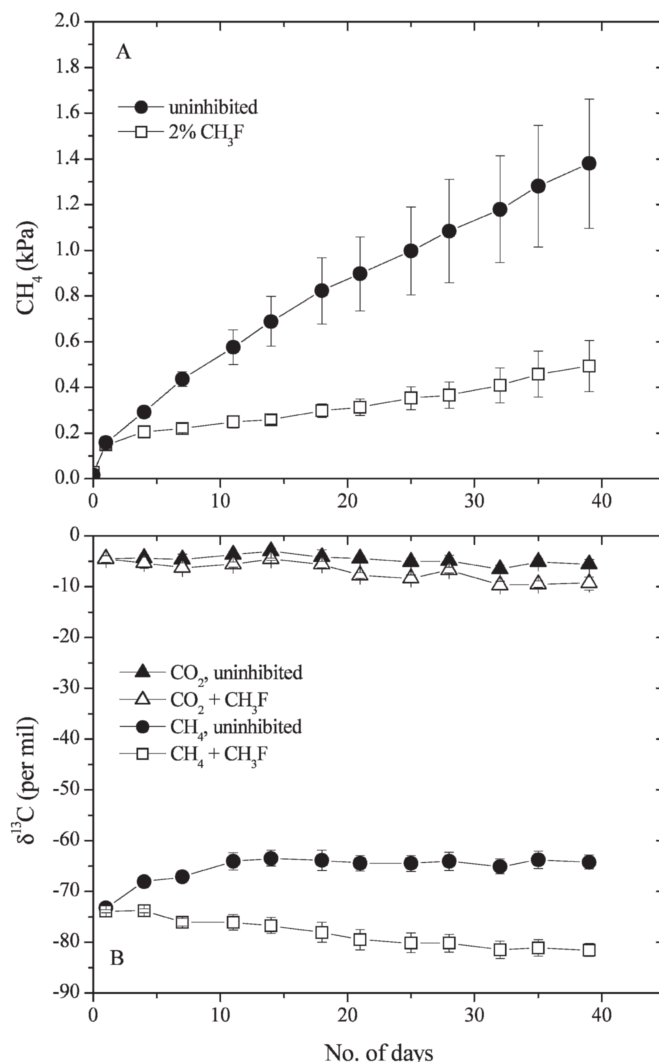


Fig. 3. Potential methane production at 30°C in the lower layers (20–25 cm depth) of profundal sediment of Lake Stechlin in the absence (closed symbols) and presence (open symbols) of methyl fluoride; (A) accumulation of CH_4; (B) $\delta^{13}C$ of the accumulated CH_4 and CO_2 ; mean ± SE, n = 3.

The ΔG^0 values were corrected for the actual temperature using the Van't Hoff equation. The actual ΔG at the incubation conditions were calculated from these temperature-corrected ΔG^0 and the actual partial pressures of CH_4, CO_2 and H_2 and the actual concentrations of acetate and H^+ (pH 7.4) with the Nernst equation.

Results

The concentrations of dissolved CH_4, nitrate, and sulfate in the profundal sediment are shown in Fig. 1. The pH was 7.2–7.4. Sulfate was effectively depleted below 10 cm depth with residual concentrations of about 20–40 μmol L^{-1}. Methane was not detectable in the water column and in the upper surface layers, but increased linearly below 2 cm depth. Similar profiles have been observed before (Casper et al. 2003; Sass et al. 2003). In the hypolimnetic water adjacent to the sediment surface at 32 m water depth was a water temperature of 5.6°C and 56% O_2 saturation.

Because CH_4 production rates in the sediment at in situ temperature were extremely low, we primarily measured potential rates at an elevated temperature of 30°C. The CH_4 production potential was measured in an upper (0–5 cm) and a lower (20–25 cm) sediment layer. In the upper sediment samples, CH_4 accumulated after a lag phase of about 15 days (Fig. 2A). The initially present sulfate was depleted within 1 day to <5 μmol L^{-1}. Subsequently, methanogenesis became increasingly active reaching a constant rate at about day 15. In the lower sediment samples, where sulfate was not available, CH_4 production started right from the beginning (Fig. 3A), albeit at a 10-times lower rate than in the upper sediment (Table 1).

Partial pressures of H_2 in the incubations of the upper sediment increased rapidly after sulfate had been depleted,

Table 1. Rates of CH_4 production in profundal sediment of Lake Stechlin in the presence and absence of CH_3F; given as mean ± SE of triplicate incubations.

Depth (cm)	Temperature (°C)	Inhibitor	CH_4 production (nmol h^{-1} cm^{-3})	Relative activity (%)
0–5	30	Uninhibited	17.3 ± 1.3	100.0
		2% CH_3F	3.2 ± 0.2	18.7
20–25	30	Uninhibited	1.66 ± 0.31	100.0
		2% CH_3F	0.61 ± 0.22	36.5

reached a maximum after 7 days, but then dropped again until about 18 days, after which H_2 had reached values of <10 Pa (Fig. 4A). Such a transient increase of H_2 at the onset of methanogenesis has also been observed in other aquatic systems in which sulfate reduction precedes methanogenesis, such as marine sediments (Hoehler et al. 1999) or rice field soils (Yao and Conrad 1999). In the lower sediment, on the other hand, H_2 partial pressures were initially very low but eventually increased to values of about 0.2 Pa as CH_4 production progressed (Fig. 5A). The H_2 partial pressures were used for calculation of the ΔG for hydrogenotrophic methanogenesis, which are shown in Figs. 4B and 5B, respectively. ΔG was calculated from the partial pressures of H_2, CH_4, and CO_2 measured in the

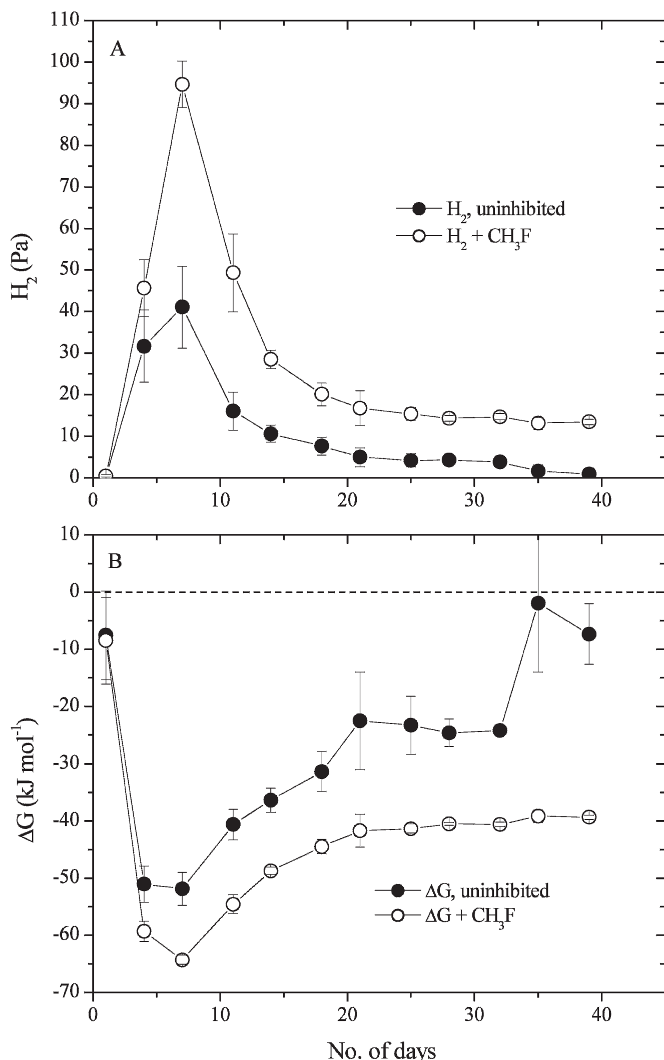


Fig. 4. (A) Partial pressures of H_2 and (B) Gibbs free energies of hydrogenotrophic methanogenesis at 30°C in the upper layers (0–5 cm depth) of profundal sediment of Lake Stechlin in the absence (closed symbols) and presence (open symbols) of methyl fluoride; mean ± SE, n = 3.

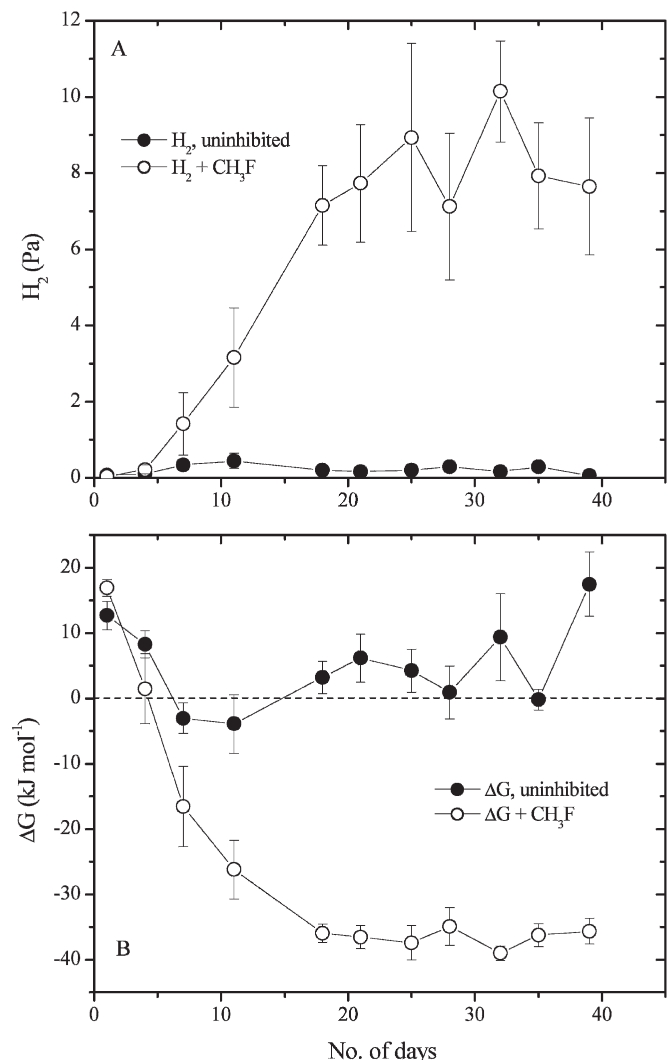


Fig. 5. (A) Partial pressures of H_2 and (B) Gibbs free energies of hydrogenotrophic methanogenesis at 30°C in the lower layers (20–25 cm depth) of profundal sediment of Lake Stechlin in the absence (closed symbols) and presence (open symbols) of methyl fluoride; mean ± SE, n = 3.

Table 2. Calculation of f_{mc} from stable isotope signatures in sediment of Lake Stechlin.

Depth (cm)	Temperature (°C)	δ_{CH4} (‰)*	δ_{mc} (‰)*	$\delta_{ac\text{-}methyl}$ (‰)†	Fraction of CH_4 formed from $H_2 + CO_2$ (f_{mc}) (%)‡ A	B	C
0–5	30	−54.26 ± 5.67	−81.73 ± 1.64	−35.55	42 ± 13	26 ± 17	14 ± 19
20–25	30	−63.85 ± 4.72	−85.04 ± 2.57	−36.29	55 ± 7	43 ± 10	34 ± 12

* Newly formed CH_4 calculated with Eq. 2, given as mean ± SE of the last five time points.
† Mean value of triplicates in the presence of CH_3F at the end of the incubations (compare with Table 3).
‡ f_{mc} calculated from δ_{CH4}, δ_{mc}, $\delta_{ac\text{-}methyl}$, and ε_{ma} with Eqs. 4 and 7 and assuming (A) ε_{ma} = 0, (B) ε_{ma} = −10‰, (C) ε_{ma} = −15‰.

headspace after the bottles had been vigorously shaken to equilibrate gas and aqueous phases. Hence, the partial pressures should be representative for the conditions in solution at the time of sampling. Values of ΔG for hydrogenotrophic homoacetogenesis were calculated only for the end of incubation (acetate concentrations were only available for this time point); these ΔG values were generally positive (>+16 kJ mol^{-1} acetate).

The carbon in the upper sediment had $\delta^{13}C$ values of C_{tot} = −21.33 ± 0.10‰, C_{org} = −25.74 ± 0.04‰, and C_{carb} = +4.41 ± 0.11‰. The carbon in the lower sediment had $\delta^{13}C$ values of C_{tot} = −24.67 ± 0.42‰, C_{org} = −25.11 ± 0.17‰, and C_{carb} = +0.44 ± 0.46‰. Hence, δ_{org} was almost the same at both depths. The $\delta^{13}C$ of CH_4, which was initially about −70 to −65‰ in the upper sediment, increased during the initial phase of active methanogenesis to about −50‰ (Fig. 2B). The δ_{CO2}, on the other hand, initially increased from about −25 to −15‰ and then stayed constant (Fig. 2B). In the lower sediment, the δ_{CH4} also increased during the initial phase of methanogenesis from −75 to −65‰, whereas δ_{CO2} stayed at a constant value of about −5‰ (Fig. 3B). The $\delta^{13}C$ of newly formed CH_4, which was calculated from time point to time point, was on the average, −54‰ and −64‰ for the upper and lower sediment, respectively, during the linear phase of CH_4 production (the last five time points) (Table 2).

The sediment samples were also incubated in the presence of 2% methyl fluoride (CH_3F), a specific inhibitor of acetoclastic methanogenesis (Frenzel and Bosse 1996; Janssen and Frenzel 1997). The applied concentration had been found to completely inhibit acetoclastic methanogenesis in the sediment of eutrophic Lake Dagow (Chan et al. 2005). Addition of CH_3F resulted in partial inhibition of CH_4 production (Figs. 2A, 3A), with residual activities of 19–36% (Table 1). Partial pressures of H_2 were higher in the presence of CH_3F, resulting in significantly lower ΔG for hydrogenotrophic methanogenesis (Figs. 4, 5), but ΔG for hydrogenotrophic homoacetogenesis was still positive (>+4 kJ mol^{-1} acetate). Addition of CH_3F also strongly affected the $\delta^{13}C$ of the accumulating CH_4, which generally decreased to much lower values (−90 to −80‰) than in the uninhibited sediment (Figs. 2B, 3B; Table 2). The δ_{CO2}, on the other hand, was comparatively little affected by the addition of CH_3F (Figs. 2B, 3B).

The concentrations of acetate in the uninhibited sediment were very low (<35 μmol L^{-1}). However, on inhibition of acetoclastic methanogenesis with CH_3F, acetate accumulated to millimolar concentrations (Table 3). The amount of accumulated acetate was fairly stoichiometric to the amount of CH_4 that was not produced because of inhibition and was larger in the upper (155 ± 20 and 200 ± 9 μmol, respectively) than in the lower (40 ± 13 and 32 ± 11 μmol, respectively) sediment layers. The accumulation of acetate allowed the determination of the $\delta^{13}C$ in total acetate and acetate-methyl at the end of the incubation (Table 3). At both sediment depths, $\delta_{ac\text{-}methyl}$ was about −35‰ to −36‰. In the uninhibited sediment samples, the amount of acetate was too low to allow determination of $\delta^{13}C$ in the acetate-methyl. However, when the triplicate samples were pooled, determination was possible, giving a single value that was almost equal to the average measured in the CH_3F-inhibited samples (Table 3).

Molecular analyses were done in sediment that was immediately frozen after sampling, thus targeting the microbial community in situ. The methanogenic microbial community in the upper and lower sediment was analyzed by cloning (111 and 100 clones, respectively) and sequencing of archaeal 16S rRNA gene fragments. The clones were grouped into different OTUs according to the banding position in DGGE analysis. Representative clones of each OTU were sequenced and phylogenetically positioned (Fig. 6). The following archaeal lineages were detected: the methanogenic lineages of *Methanomicrobiales* and *Methanosaetaceae* and the (probably) nonmethanogenic lineages of the LDS cluster (Glissmann et al. 2004), Rice cluster V (Grosskopf et al. 1998*b*), and Group III (DeLong

Table 3. Stable isotopic signature in the acetate accumulated in profundal sediment of Lake Stechlin in the presence and absence of CH_3F; mean values ± SE at the end of triplicate incubations.

Depth (cm)	Temperature (°C)		Acetate (μmol L^{-1})	$\delta^{13}C$ of total acetate (‰)	$\delta^{13}C$ of acetate-methyl (‰)	$\delta^{13}C$ of acetate-carboxyl (‰)
0–5	30	+CH_3F	6,330 ± 880	−23.99 ± 0.36	−35.55 ± 2.07	−12.43 ± 1.35
		Uninhibited	34 ± 6	−23.31 ± 0.85	−35.81	−10.81
20–25	30	+CH_3F	1,570 ± 495	−25.70 ± 1.00	−36.29 ± 1.73	−15.11 ± 0.29
		Uninhibited	35 ± 2	−25.59 ± 0.71	Samples lost	Samples lost

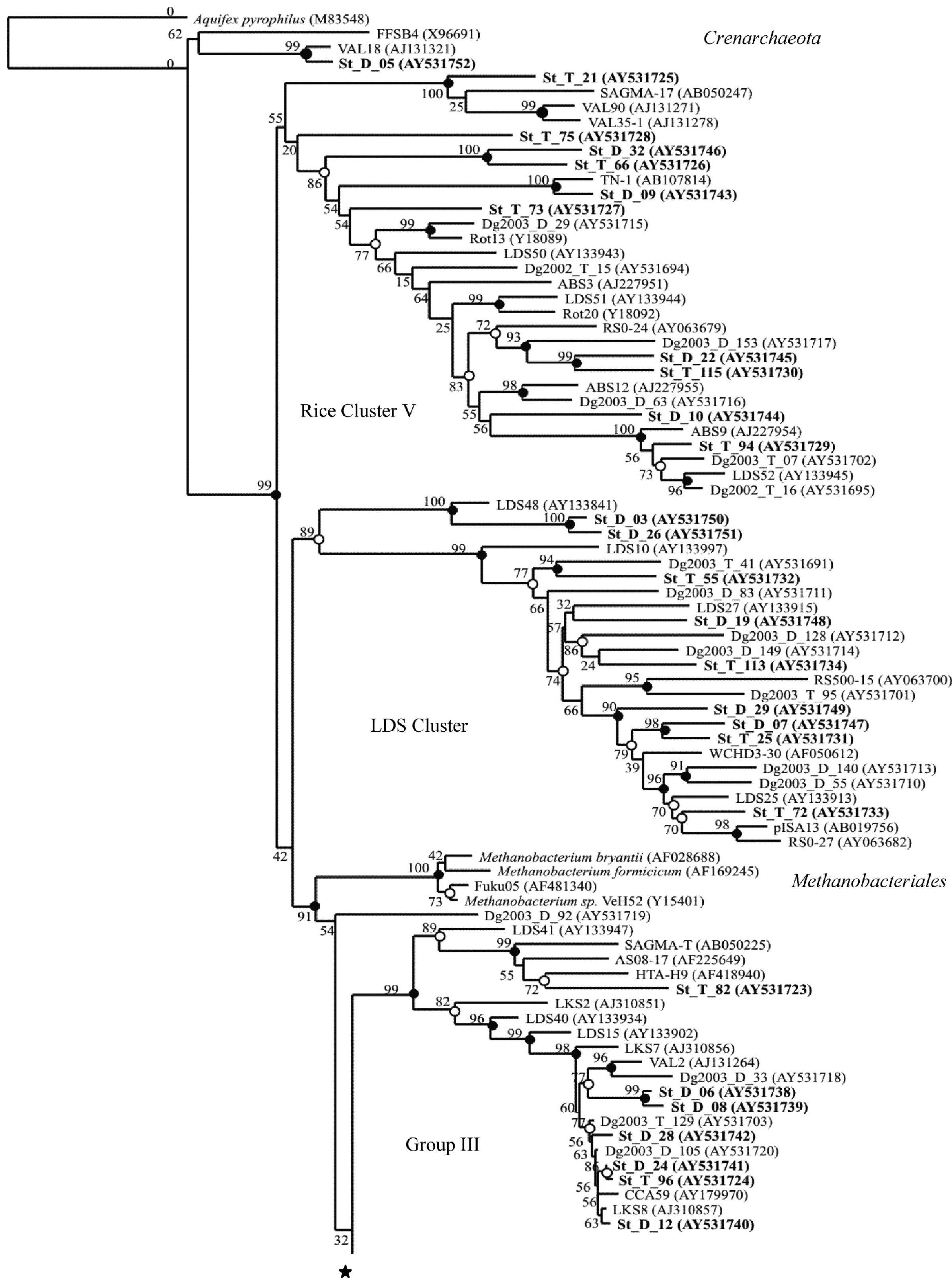


Fig. 6. Phylogenetic relationship of archaeal 16S rRNA gene clone sequences analyzed by maximum likelihood algorithm. The sequences retrieved from Lake Stechlin sediment are shown in bold. Bootstrap values are indicated (closed circles >90%; open circles >70%). The scale bar indicates 10% sequence divergence. The designations of the clones retrieved from Lake Stechlin sediment have the following meanings: T, 0–5 cm depth; D, 20–25 cm depth.

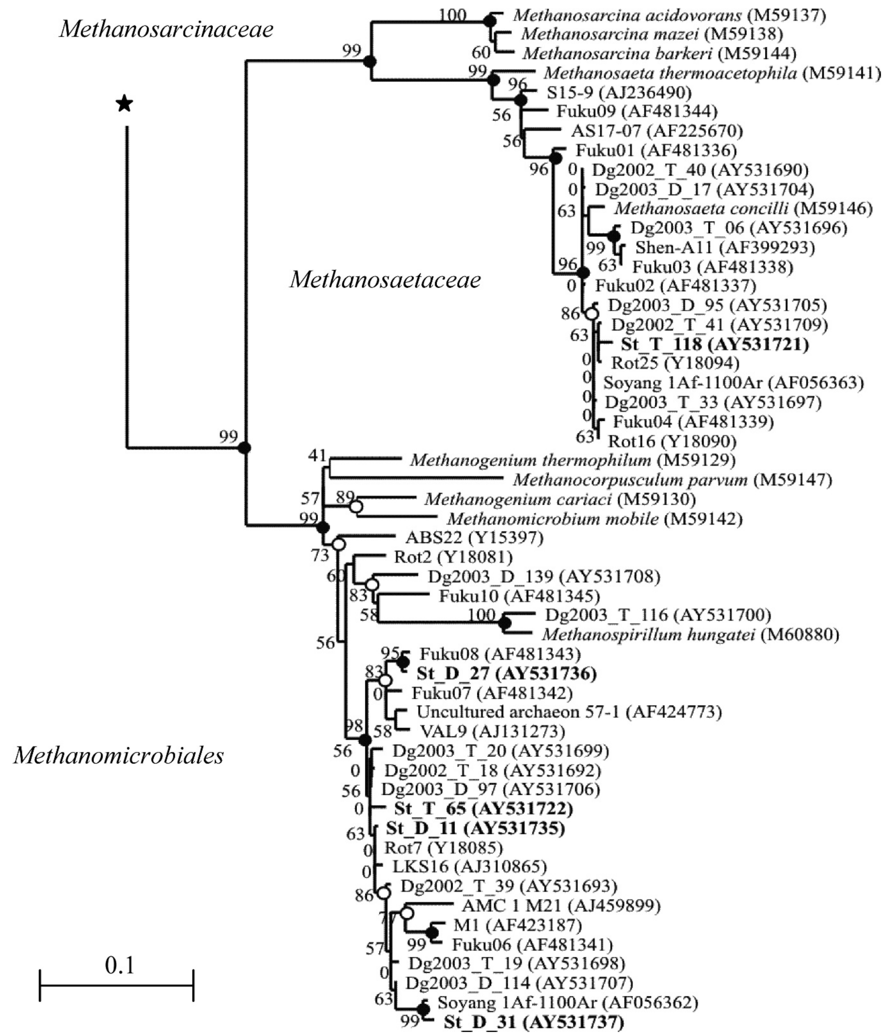


Fig. 6. Continued.

and Pace 2001). *Methanosarcinaceae* and *Methanobacteriales* were not detected. The relative contribution of each OTU to the total number of clones is shown in Table 4. In the upper sediment, *Methanosaetaceae* and *Methanomicrobiales* contributed each only 7–8% to the total clones, the rest being made up by other nonmethanogenic Archaea. In the lower sediment, the only methanogenic Archaea detectable were *Methanomicrobiales*, albeit at a total 32%.

In the upper sediment, the numbers of bacterial and archaeal 16S rRNA gene copies per gram of fresh sediment were $(10.3 \pm 2.4) \times 10^9$ and $(6.8 \pm 1.4) \times 10^7$, respectively. In the lower sediment, the numbers of Archaea were similar (i.e., $[7.6 \pm 0.4] \times 10^7$), but those of bacteria were an order of magnitude lower (i.e., $[1.1 \pm 0.3] \times 10^9$, compared with the upper sediment.

Discussion

In oligotrophic Lake Stechlin, methane is only produced in the deeper layers of the sediment, but not in the surface layers where sulfate reduction predominates (Fig. 1). Potential CH_4 production in the surface layers could only be detected after a lag phase, during which residual sulfate was depleted. The surface sediment of oligotrophic lakes is usually oxic and contains relatively high concentrations of sulfate or other electron acceptors, which suppress methanogenesis. After expression, potential CH_4 production was about 10 times higher in the upper than in the lower sediment layers. The different CH_4 production potentials are reflected in the number of bacteria, which were also about 10 times higher in the upper than in the lower sediment. The numbers of Archaea were similar at both depths, although the relative abundance of clones representing methanogens among the Archaea seemed to be higher in the lower than in the upper sediment. Similar patterns of bacterial and archaeal numbers have been observed in other lake sediments (Zepp-Falz et al. 1999; Chan et al. 2005), including mesotrophic Lake Biwa (Koizumi et al. 2003). Overall, potential CH_4 production was limited by the bacterial rather the archaeal component

Table 4. Frequency of individual operational taxonomic units (OTUs) of archaeal 16S rRNA gene clones retrieved from the upper and the lower sediment layers of Lake Stechlin. The randomly selected clones were differentiated by DGGE into different OTUs, of which at least one representative was sequenced and phylogenetically analyzed (indicated by the name of the clone, see Fig. 6).

	Upper sediment layer n = 111, depth 0–5 cm			Lower sediment layer n = 100, depth 20–25 cm		
Phylogenetic group	OTU	Frequency (%)	Subtotal (%)	OTU	Frequency (%)	Subtotal (%)
Methanosaetaceae	St_T_118	7		n.d.	0	
			7			0
Methanomicrobiales	St_T_65	8		St_D_11	13	
				St_D_27	8	
				St_D_31	11	
			8			32
Group III	St_T_82	5		St_D_06	2	
	St_T_96	6		St_D_08	3	
				St_D_12	13	
				St_D_24	8	
				St_D_28	11	
			11			37
Rice cluster V	St_T_21	11		St_D_09	2	
	St_T_66	7		St_D_10	4	
	St_T_73	5		St_D_22	3	
	St_T_75	4		St_D_32	3	
	St_T_94	5				
	St_T_115	9				
			41			12
LDS cluster	St_T_25	10		St_D_07	2	
	St_T_55	9		St_D_19	3	
	St_T_72	8		St_D_29	4	
	St_T_113	6		St_D_03	3	
				St_D_26	3	
			33			15
Crenarcheota	n.d.	0		St_D_05	4	
			0			4

n.d., not detected.

of the microbial community, which is reasonable because the initial steps of the degradation of organic matter, which are catalyzed by hydrolytic and fermenting bacteria, are usually the rate-limiting steps for CH_4 production (Billen 1982). It is interesting that a sufficiently active population of methanogenic Archaea was preserved in the upper sediment layers, although under in situ conditions CH_4 production should never be expressed because of competing sulfate reduction driven by sulfate concentrations $>100\ \mu mol\ L^{-1}$ in the upper sediment layer throughout the year. It is noteworthy that CH_4 production in the upper sediment (assayed at 30°C) was only expressed after a lag phase, and that this lag lasted almost 2 weeks after complete depletion of sulfate. At close to in situ temperature (4°C), the CH_4 production potential was also expressed, albeit after a much longer lag phase and at a much lower rate (unpublished results). The methanogens in the upper sediment apparently were not in an active state and required time to become active, whereas those in the lower sediment were active right from the beginning, but exhibited a much lower CH_4 production rate than in the upper sediment. This pattern is probably driven not only by the availability of sulfate in the two sediment horizons, but also by the availability of electron donors, which is presumably higher in the upper than the lower sediment.

The overall composition of archaeal 16S rRNA sequences was very similar to that found in other freshwater lakes (Zepp-Falz et al. 1999; Nüsslein et al. 2001; Glissmann et al. 2004). Interestingly, 16S rRNA gene sequences of acetoclastic *Methanosaeta* spp. were only detected in the upper but not in the lower sediment, whereas hydrogenotrophic *Methanomicrobiales* were found at both depths. The function of the presumably nonmethanogenic Archaea (Group III, LDS cluster, and Rice cluster V) for sediment biogeochemistry is presently unknown. In fact, it is surprising that the upper sediment layers contain such a diverse community of methanogenic groups, although methane production hardly operates in these layers, which are dominated by sulfate reduction under in situ conditions (Sass et al. 2003).

Note that size and composition of the microbial community represent in situ conditions. The incubation experiments at 30°C showed that these methanogenic communities were potentially active after sulfate was reduced. We presently do not understand how the generally oxidized surface sediments became colonized by methanogenic Archaea in the first place because colonization depends on generating energy by CH_4 production. Input by sedimentation is unlikely because the water column is oxic and not supportive of methanogenesis. Invasion from

deep sediment is also unlikely because the surface sediment is normally dominated by sulfate reduction and not supportive for methanogenesis either. Also the question remains, how under such conditions the deep sediment would have become colonized by methanogens because it is nothing but surface sediment that was eventually buried, probably >100 years ago. We speculate that the surface sediment is supportive of methanogenesis at least locally (e.g., within anoxic microniches that do not contribute much to overall biogeochemistry under in situ conditions). Such niches could be created by the annual deposition of fresh particulate matter after the algal bloom. However, this speculation requires further research.

Besides quantifying methanogenic populations and rates of CH_4 production, we also investigated stable isotope biogeochemistry, the path of CH_4 production, and the Gibbs free energies of CH_4 productions from $H_2 + CO_2$ in the upper versus the lower sediment layers incubated at 30°C. Production of CH_4 was partially inhibited by CH_3F, an inhibitor of acetoclastic methanogenesis (Frenzel and Bosse 1996; Janssen and Frenzel 1997). Because acetate accumulated stoichiometrically compared with inhibited CH_4 production, fermentative production of acetate was apparently balanced by methanogenic consumption. Compared with similar experiments in other aquatic environments (e.g., Chan et al. 2005), it is reasonable to assume that the applied CH_3F concentration was sufficient to inhibit acetoclastic methanogenesis completely. Although hydrogenotrophic methanogens compared with acetoclastic methanogens are not very sensitive to CH_3F, it is likely that they also became partially inhibited at the CH_3F concentration applied. This conclusion is based on the observation that H_2 partial pressures were higher, and thus ΔG values were lower, in the presence of CH_3F (Figs. 4, 5), indicating that H_2 consumption was no longer as efficient as in uninhibited sediment (i.e., hydrogenotrophic methanogenesis was partially inhibited). Therefore, residual activities of CH_4 production upon inhibition (Table 1) do not give robust values for the relative contribution of hydrogenotrophic methanogenesis to total CH_4 production.

However, the δ_{CH4} of the residual CH_4 production should exclusively represent the signature of hydrogenotrophic methanogenesis (δ_{mc}) (Table 2), but not that of acetoclastic methanogenesis (δ_{ma}). Likewise, the $\delta^{13}C$ of the acetate accumulating in the presence of CH_3F ($\delta_{ac\text{-}methyl\text{-}CH3F}$) should exclusively represent the isotopic signature of the acetate that was newly formed without the effect exerted by the consumption of acetate. Table 2 also gives the $\delta_{ac\text{-}methyl}$ at the end of incubation and the fraction of hydrogenotrophic CH_4 production (f_{mc}) calculated thereof. It is possible to calculate f_{mc} from δ_{CH4}, δ_{mc}, and δ_{ma} with Eq. 4. Values of δ_{ma} cannot be measured directly, however, they are related to values of $\delta_{ac\text{-}methyl}$ by

$$\delta_{ma} = \delta_{ac-methyl} + \varepsilon_{ma} \quad (7)$$

with δ_{ma} being the isotopic enrichment factor for the conversion of acetate-methyl to methane. For steady state between acetate production and consumption, we can calculate δ_{ma} from Eq. 7 by assuming $\varepsilon_{ma} = -15‰$ to $-10‰$ (Table 3). These values are reasonable for the genus *Methanosaeta* (Valentine et al. 2004; Penning et al. 2006*a*), to which the acetate-consuming methanogens detected in the sediment belong exclusively (see above). The thus calculated values of f_{mc} (Table 3) were lower in the upper than in the deeper sediment. For $\varepsilon_{ma} = -15‰$ they were <33%, which is theoretically expected when organic matter (e.g., polysaccharides) is degraded under methanogenic conditions (Conrad 1999). However, ongoing fractionation by acetoclastic methanogens with $\varepsilon_{ma} = -15‰$ should have resulted in isotopic enrichment of acetate-methyl in the control versus the CH_3F-inhibited incubations, which was not the case (Table 3). Such enrichment has been observed during methanogenesis on rice roots (Penning et al. 2006*b*) but was not evident in the incubations of Lake Stechlin sediment. Therefore, it is unlikely that acetate-methyl was fractionated during the further conversion to CH_4.

Because acetate concentrations in uninhibited sediment were very low (<35 $\mu mol\ L^{-1}$), one could alternatively assume that the produced acetate was quantitatively consumed so that $\varepsilon_{ma} = 0$ and $\delta_{ma} = \delta_{ac\text{-}methyl\text{-}CH3F}$ (Table 2). By assuming $\varepsilon_{ma} = 0$, f_{mc} was 42% and 55% in the upper and lower sediment, respectively (Table 2). The values of f_{mc} were slightly higher in the lower than in the upper sediment layers, which is reasonable because, in the lower sediment layers, only hydrogenotrophic, but no acetoclastic methanogens were detected. Nevertheless, acetoclastic methanogenesis also occurred in the deeper sediment layers, accounting for about 45% of the total (Table 2). Because clones assigned to *Methanosaeta* were not very common in the upper sediment layers (comprising only 7% of the total archaeal clones), we do not exclude having failed to detect acetoclastic methanogens in the deeper sediment that might have been present. The f_{mc} values were relatively high (>42%). Such high values have occasionally been found in nature, but the mechanistic basis for them is not always clear (Conrad 1999). A possible explanation is that acetate was mainly consumed by syntrophic acetate oxidation rather than acetoclastic methanogenesis, during which acetate is first converted fermentatively to CO_2 and H_2, which is then converted to CH_4 by hydrogenotrophic methanogenesis. For this process, acetoclastic *Methanosaeta* spp. are not required. Evidence for this process, which results in a relatively large f_{mc}, has been found in sediment of Lake Kinneret (Nüsslein et al. 2001, 2003). Basically, this process is the reverse of homoacetogenesis from $H_2 + CO_2$. Because the ΔG of homoacetogenesis was positive in the sediment incubations at 30°C, the ΔG of syntrophic acetate oxidation would have been negative and thus thermodynamically feasible. We do not know the isotopic fractionation factor of syntrophic acetate oxidation and therefore cannot exclude that the isotopic signature of CH_4 would be compatible with such a process. However, because of the similarity of isotopic signatures, we would have to assume that it occurred both in the upper and the lower sediment layers.

With the knowledge of δ_{mc} and δ_{CO2} and with the use of Eq. 3 we can calculate α_{mc}, the isotopic fractionation factor

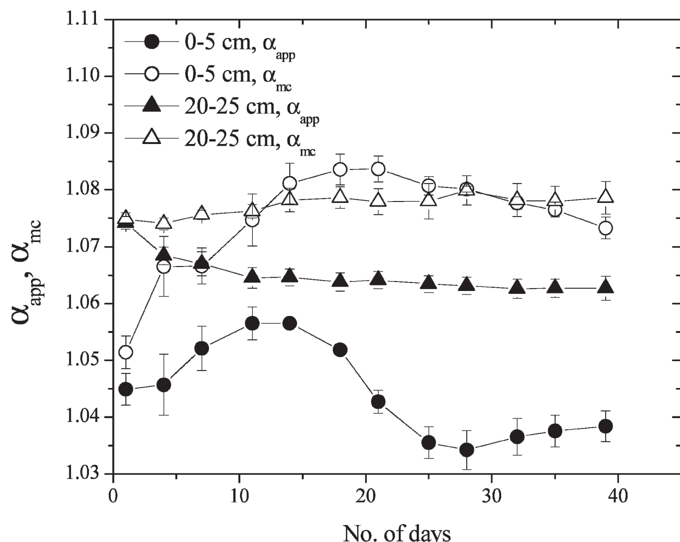


Fig. 7. Isotopic fractionation factors (α) for the conversion of CO_2 to CH_4 in the upper and lower layers of the profundal sediment of Lake Stechlin. Values of α_{app} and α_{mc} were calculated with Eq. 3 and $\delta^{13}C$ data (shown in Figs. 2 and 3) measured in uninhibited (closed symbols) and CH_3F-inhibited (open symbols) incubations, respectively.

for hydrogenotrophic methanogenesis (reduction of CO_2 to CH_4). The temporal change of α_{mc} values is shown in Fig. 7 together with that of α_{app}, which is calculated from δ_{CH4} instead of δ_{mc}. As expected, values of α_{mc}, which exhibit the isotopic signal of δ_{mc} only, were generally higher than those of α_{app}, which exhibit the isotopic signal of both δ_{mc} and δ_{ma}. The α_{mc} are at the upper end of values reported in the literature for cultures of hydrogenotrophic methanogens (Conrad 2005). Values of α_{mc} were shown to depend on the energetic conditions for hydrogenotrophic methanogens (i.e., the Gibbs free energy, ΔG; Penning et al. 2005). Therefore, with the relationship of α_{mc} versus ΔG published by Penning et al. (2005), we were able to calculate ΔG values from the α_{mc}. The thus obtained ΔG were about -45 kJ mol^{-1} for both sediment depths (Table 5). The ΔG values derived from measured H_2 partial pressures, on the other hand, were slightly positive in the uninhibited lower sediment and about -20 kJ mol^{-1} in the uninhibited upper sediment (Table 5), which are below or at the energetic threshold of hydrogenotrophic methanogenesis, respectively (Conrad 1999; Hoehler et al. 2001). The ΔG values derived from H_2 partial pressures measured in CH_3F-inhibited sediment, however, were similar to those calculated from α_{mc} (Table 5). Note that α_{mc} was also determined under CH_3F-inhibited conditions. Analyzing various wetland environments, Penning et al. (2005) found that ΔG values derived from α_{mc} were often substantially more negative than ΔG values derived from H_2 partial pressures measured under CH_3F-inhibited conditions. These authors concluded that values of α_{mc} might represent a better proxy for in situ energetic conditions than H_2 partial pressures because they are measured in the gas headspace of sediment incubations and might not represent real in situ conditions, since H_2 concentrations notoriously exhibit steep concentration gradients in the environment (Hoehler et al. 2001). In this study, we obtained similar ΔG values with the use of α_{mc} or H_2 partial pressures, provided CH_3F was applied as inhibitor. Without inhibitor, however, H_2 partial pressures gave unrealistically high, even positive, ΔG values. We assume that energetic conditions for hydrogenotrophic methanogenesis were more favorable in situ than suggested by the measured H_2 partial pressure. This result is in agreement with the existence of anoxic microniches within the sediment in which H_2 might be produced in the vicinity of H_2-consuming methanogens, but not completely equilibrated with the headspace of the incubation.

In the sediment incubations at 30°C, the $\delta^{13}C$ in the newly formed acetate-methyl was about -35‰ (Table 2), which is lighter than the $\delta^{13}C$ in the sediment organic carbon by 10‰. A δ_{org} of -25‰ is characteristic for C3 photosynthesis (O'Leary 1981) and reflects a weighted balance of the main sedimenting materials, like phytoplankton algae, terrestrial leaves, and macrophytes (Casper et al. 1993). However, the isotopic depletion by -10‰ during formation of acetate is unusually large. Isotopic fractionation during bacterial fermentation of organic matter to acetate is usually negligible or results in small (+3‰) isotopic enrichment (Blair et al. 1985; Penning and Conrad 2006). A reasonable explanation for a larger isotope effect would be the operation of chemolithotrophic homoacetogenesis (i.e., the formation of acetate from H_2 + CO_2), which exhibits a relatively large enrichment factor of $\delta_{CO2\text{-}ac} = -59$‰ (Gelwicks et al. 1989). Hydrogenotrophic homoacetogenesis occurs in aquatic sediments if the H_2 partial pressure is sufficiently high (Hoehler et al. 1999). However, this is an unlikely explanation for the low $\delta^{13}C$ of acetate-methyl observed in Lake Stechlin sediment because

Table 5. Fractionation factors and Gibbs free energies of hydrogenotrophic methanogenesis in profundal sediment of Lake Stechlin.

			ΔG (kJ mol^{-1})		
				From H_2 partial pressures‡	
Depth (cm)	Temperature (°C)	α_{mc}*	From α_{mc}†	Uninhibited	With CH_3F^c
0–5	30	1.078 ± 0.001	−44.5	−20.3 ± 3.1	−40.3 ± 0.4
20–25	30	1.078 ± 0.001	−44.5	+2.4 ± 1.2	−37.3 ± 0.6

* Values of α_{mc} are the mean ± SE of the last five time points in Fig. 7.
† ΔG was calculated from α_{mc} according to the theoretical relationship between ΔG and α_{mc} (Penning et al. 2005).
‡ Values of ΔG are the mean ± SE of the last five time points in Figs. 4 and 5.

the ΔG of this process was not negative. Furthermore, the large intramolecular difference of $\delta_{ac\text{-}carboxyl} - \delta_{ac\text{-}methyl}$ of about 21–25‰ (calculated from Table 3) also contradicts chemolithotrophic homoacetogenesis that has not such an intramolecular difference (Gelwicks et al. 1989). By contrast, such a large intramolecular difference is similar to that observed in acetate produced by fermentation of saccharides (~23‰) (Blair et al. 1985). The isotopic data thus do not provide a conclusive explanation for the relatively large depletion of ^{13}C during conversion of organic carbon to acetate. We speculate that fermenting bacteria might exist that fractionate carbon isotopes differently from those studied so far (Blair et al. 1985; Penning and Conrad 2006).

Our experiments have shown that the upper sediment layers of an oligotrophic lake contain a considerable diversity of methanogenic Archaea and a large capacity for methanogenesis when compared with deeper sediment layers. A full analysis of $\delta^{13}C$ in organic carbon, acetate, CO_2, and CH_4 proved to be useful for the determination of the isotopic fractionation factors involved in methanogenesis and the quantification of the physiological paths contributing to CH_4 production. It furthermore allowed a rather comprehensive quantification of isotopic fractionation during fermentative acetate production and during methane production from CO_2 and acetate, in particular because isotopic signatures for the methyl group of acetate are reported, which have rarely been studied before in natural aquatic systems (Blair and Carter 1992; Sugimoto and Wada 1993; Gelwicks et al. 1994). The results indicate substantial fractionation of carbon isotopes during acetate formation from organic matter, which is presently not understood on the basis of bacterial physiology. Hence, it appears that the upper sediment layers of oligotrophic lakes contain an interesting methanogenic community whose ecological relevance is not fully understood. In particular, it is surprising how such a community has established and maintained itself in the surface sediment, where it is normally not expressed under in situ conditions because of the dominance of sulfate reduction. Analysis of stable isotopes under in situ conditions (low temperature), similar to the sediment incubations done in this study at elevated temperature, might be a useful approach to learning more about methane biogeochemistry in oligotrophic lake sediments.

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