View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by RERO DOC Digital Library

RESEARCH ARTICLE

Influence of temperature and high acetate concentrations on methanogenesis in lake sediment slurries

Alla N. Nozhevnikova[1,2], Valeria Nekrasova[1], Adrian Ammann[3], Alexander J.B. Zehnder[3,4], Bernhard Wehrli[2] & Christof Holliger[2,5]

[1]Institute of Microbiology Russian Academy of Sciences, Moscow, Russia; [2]Swiss Federal Institute for Environmental Sciences and Technology (EAWAG), Kastanienbaum, Switzerland; [3]Swiss Federal Institute for Environmental Science and Technology (EAWAG), Ueberlandstrasse, Duebendorf, Switzerland; [4]Board of the Swiss Federal Institutes of Technology, Zurich, Switzerland; and [5]Ecole Polytechnique Fédérale de Lausanne (EPFL), Laboratory for Environmental Biotechnology, Lausanne, Switzerland

Correspondence: Christof Holliger, EPFL-LBE, CH03425, Station 6, 1015 Lausanne, Switzerland. Tel.: +4 121 693 4724; fax: +4 121 693 4722; e-mail: christof.holliger@epfl.ch

Received 15 May 2007; revised 14 August 2007; accepted 20 August 2007.
First published online November 2007.

DOI:10.1111/j.1574-6941.2007.00389.x

Editor: Alfons Stams

Keywords
acetogenesis; acetotrophic; hydrogenotrophic methanogenesis; psychrophilic; mesophilic; thermophilic methanogenic microbial community.

Abstract

Methanogenesis from main methane precursors H_2/CO_2 and acetate was investigated in a temperature range of 2–70 °C using sediments from Lake Baldegg, Switzerland. Psychrophilic, psychrotrophic, mesophilic, and thermophilic methanogenic microbial communities were enriched by incubations for 1–3 months of nonamended sediment slurries at 5, 15, 30, and 50 °C. Isotope experiments with slurries amended with ^{14}C-labeled bicarbonate and ^{14}C-2-acetate showed that in the psychrophilic community (enriched at 5 °C), about 95% of methane originated from acetate, in contrast to the thermophilic community (50 °C) where up to 98% of methane was formed from bicarbonate. In the mesophilic community (30 °C), acetate was the precursor of about 80% of the methane produced. When the hydrogen–carbon dioxide mixture (H_2/CO_2) was used as a substrate, it was directly converted to methane under thermophilic conditions (70 and 50 °C). Under mesophilic conditions (30 °C), both pathways, hydrogenotrophic and acetoclastic, were observed. At low temperatures (5 and 15 °C), H_2/CO_2 was converted into methane by a two-step process; first acetate was formed, followed by methane production from acetate. When slurries were incubated at high partial pressures of H_2/CO_2, the high concentrations of acetate produced of more than 20 mM inhibited acetoclastic methanogenesis at a temperature below 15 °C. However, slow adaptation of the psychrophilic microbial community to high acetate concentrations was observed.

Introduction

Profundal sediments of deep stratified lakes are permanent low-temperature environments. The major electron acceptor present for the biological oxidation of organic matter in the anoxic sediments of these lakes is carbon dioxide, leading to the production of methane. Methanogenesis at low temperatures in different lake sediments has repeatedly been shown to occur (Zeikus & Winfrey, 1976; Zehnder & Brock, 1980; Kelly & Chynoweth, 1981; Jones *et al.*, 1982; Conrad *et al.*, 1989; Nozhevnikova *et al.*, 1997, 2003; Zepp-Falz *et al.*, 1999; Casper *et al.*, 2003). Strong evidence that specific psychrophilic methanogenic communities exist in profundal lake sediments was provided recently (Nozhevnikova *et al.*, 2003). Nevertheless, these sediments represent a unique natural reservoir of different kinds of anaerobic microorganisms because sediment slurries incubated at different temperatures produced methane in a temperature range of 2–70 °C (Nozhevnikova *et al.*, 1997, 2003).

Acetate has been proposed to be a major methane precursor under low-temperature conditions based on different experimental evidence (Kuivila *et al.*, 1989; Kotsyurbenko *et al.*, 1993; Thebrath *et al.*, 1993; Schulz & Conrad, 1996; Nozhevnikova *et al.*, 1997; Schulz *et al.*, 1997; Zepp-Falz *et al.*, 1999; Nüsslein *et al.*, 2001; Glissmann *et al.*, 2004). The prevalence of acetogenic bacteria in hydrogen consumption at a low temperature was shown in different cold environments, and several psychrophilic acetogenic bacteria were isolated from these environments (Jones & Simon, 1985; Conrad *et al.*, 1989; Kotsyurbenko *et al.*, 1993,

© 2007 Federation of European Microbiological Societies
Published by Blackwell Publishing Ltd. All rights reserved

1995, 1996, 2001; Küsel & Drake, 1995; Simankova *et al.*, 2000). Surprisingly, the only psychrophilic and psychrotrophic methanogens that have been isolated so far are hydrogenophilic ones (Franzmann *et al.*, 1992, 1997; Simankova *et al.*, 2001, 2003). A few studies contradict the finding of acetate as a major methane precursor and acetogenesis as the prevailing hydrogen-consuming process in lake sediments (Jones *et al.*, 1982; Namsaraev *et al.*, 1995; Murase & Sugimoto, 2001). As a consequence, the question on the exact quantitative role of hydrogenotrophic and acetotrophic methanogenesis in cold freshwater habitats is not yet fully answered.

It is also not yet well known how temperature influences the pathways of methane production in different methanogenic ecosystems. A shift from acetoclastic to hydrogenotrophic methanogenesis with temperature increase has been observed in anoxic rice soil (Chin & Conrad, 1995; Chin *et al.*, 1999; Fey & Conrad, 2000, 2003; Fay *et al.*, 2001, 2004; Conrad *et al.*, 2002), and with lake sediments (Schulz *et al.*, 1997; Nüsslein & Conrad, 2000; Glissmann *et al.*, 2004). The shift in incubation temperature has led to a change in the composition of the microbial community in rice field soil (Chin *et al.*, 1999; Fey & Conrad, 2000), but not in lake sediments (Glissmann *et al.*, 2004). Much less is known about the influence of main methane precursor concentrations on the rate of methanogenesis at different temperatures. Half-saturation constants (K_s) of acetate methanation have been reported to increase at a low temperature (Lin *et al.*, 1987), whereas the half-saturation constant (K_M) of acetate uptake has been found to decrease (Westermann *et al.*, 1989). A modeling study of H_2/CO_2 and acetate conversion to methane by sediment samples at a low temperature has indicated a negative effect of increasing acetate concentrations on methanogenesis (Lokshina *et al.*, 2001). An adaptation of microbial communities to high substrate concentrations is crucial in anaerobic treatment of wastewater at a low temperature (Lettinga *et al.*, 1999).

In the present study, methanogenesis was investigated from the main methane precursors H_2/CO_2 and acetate by microbial communities of deep lake sediments enriched at different temperatures in a range from 5 to 50 °C. The goal was to assess the input of methanogenesis derived from H_2/CO_2 and acetate in nonamended sediment slurries and to investigate the influence of increasing initial H_2/CO_2 and acetate concentrations on methane production.

Materials and methods

Study site, sampling, slurry preparation, and enrichment

Sediment samples were collected from Lake Baldegg at maximum depth (66 m) with a gravity corer (Kelts *et al.*, 1986). The lake is situated in central Switzerland in an area with intensive agriculture. Lake Baldegg had an anoxic hypolimnion for almost 100 years until aeration started in 1982. The sediment, however, is still anoxic (Lotter, 1989). Sediment cores were immediately closed with rubber stoppers, transported to the laboratory in cool boxes and subsequently sampled in the cold room at 6 °C in a glove box under a nitrogen atmosphere. The upper 2 cm of crumbly sediments were cut out and 2–50 cm of the each sediment core was taken, diluted two times with anaerobic mineral medium, and thoroughly mixed. The dilution medium contained (mg L^{-1}): NH_4Cl (330); $MgCl_2 \cdot 6H_2O$ (500); $CaCl_2$ (168); KCl (330); KH_2PO_4 (330); and $NaHCO_3$ (500). Sodium sulfide (0.5 g L^{-1}) was added as a reducing agent. The final pH of the medium was 7.3, and 7.0 for the slurry. The absolute dry mass (ADM) in the slurries was about 170 mg mL^{-1}, and the organic carbon content was 20 mg g^{-1} ADM. The sediment slurries were stored in 1 L bottles at 5 °C or directly distributed into smaller serum bottles for incubation experiments. The temperature of sediments in the lake was constant, about 5 °C all year around, and the active microbial community was psychrophilic (Nozhevnikova *et al.*, 2003). To enrich psychrotrophic, mesophilic, and thermophilic microbial communities, 1 L bottles were maintained at 5 and 15 °C for 3 months, $1\frac{1}{2}$ months at 30 °C, and 1 month at 50 °C. During these periods of incubation, about 1–4 mmol L^{-1} of methane was produced at the temperatures tested. Enriched slurries were used for incubation and isotope experiments.

Incubation experiments

Aliquots of 20 or 25 mL of sediment slurry were transferred into 50 or 120 mL serum bottles. The bottles were closed with butyl rubber stoppers and aluminum caps, and the gas phase was exchanged by several cycles of applying a vacuum and refilling with oxygen-free N_2. No substrate was added when endogenous methanogenesis was studied. When acetotrophic methanogenesis was studied, acetate was added from sterile stock solutions up to the final concentrations needed, from 2 to 100 mM. To study methanogenesis from hydrogen and carbon dioxide, the N_2 gas phase was replaced by H_2/CO_2 (4/1; v/v; 1.5 bar). The initial concentrations of hydrogen were established from 150 to 440 mmol L^{-1} using the serum bottles of different sizes and different volumes of sediment slurries. If methanogenesis needed to be inhibited, bromoethanesulfonic acid (BES) was added to a final concentration of 30 mM. The sediment samples were incubated statically at different temperatures from 2 to 70 °C. All experiments were conducted in triplicate. The methane produced was measured usually every 2–5 days during the first month of incubation and one to two times a month in long-term experiments.

© 2007 Federation of European Microbiological Societies
Published by Blackwell Publishing Ltd. All rights reserved

Chemical analysis

The production of methane was measured on a gas chromatograph (HRGC 51 60, Carlo Erba, Milano, Italy) equipped with a flame ionization detector (FID-40, Carlo Erba) and a megabore GS-Q column (J. & W. Scientific, Folsom) with a length of 30 m. Hydrogen was used as the carrier gas, with a flow rate of 4 mL min^{-1} at a temperature of 40 °C. Gas samples (0.2 mL) were injected using a gas-tight syringe (Hamilton). Quantification of the methane was performedusing a calibration curve prepared with a pure methane standard (0.714 μg mL^{-1}, Carbagas, Switzerland) and 5.0% and 10.0% pure methane mixtures with argon (PanGas, Switzerland). Acetate was measured by ion-exchange chromatography using a step gradient procedure with a weak eluent on a low-capacity column (Ammann & Rüttimann, 1995). The rates of methane and acetate production were calculated by analyzing linear parts of the production curves. ADM of sediments was determined after lyophilizing sediment slurries, and particle organic carbon (Corg) content was determined after treatment of absolutely dry (AD) sediment samples by 0.5 M HCl, as described (Gächter *et al.*, 1988).

Rates of endogenous methanogenesis

Methane production rates in nonamended slurries were measured in radiotracer experiments with ^{14}C-labeled methane precursors acetate and bicarbonate. Radioactive methane was measured with the liquid scintillation counter using NaOH solution to trapcarbon dioxide as described (Zehnder *et al.*, 1979). Aliquots of 12.5 mL of sediment slurry were transferred to 25 mL serum bottles, closed with butyl-rubber stoppers, and subsequently flushed with N_2 to a final pressure of 150 kPa ($n = 3$). The slurries were preincubated at the required temperature (6, 15, 30, and 50 °C) for a period of 2–4 days, during which methane production was monitored by GC measurements. When the rate of methane production became constant, either [2-^{14}C]-acetate (acetic acid sodium salt, Amersham, Rainham, UK) or $NaH^{14}CO_3$ (sodium bicarbonate, Amersham) was added to a final activity of 0.532 and 3.5 μCi mL^{-1}, respectively. Samples incubated without ^{14}C-labeled substrates served as controls; methane production was measured by GC. Just before addition of labeled substrates, the background level of acetate was measured by ion-exchange chromatography (Ammann & Rüttimann, 1995) and was between 5 and 21 μM in slurries preincubated at 6 °C and at 50 °C, respectively. The alkalinity was analyzed with the Aquamerktest kit (Merck, Dietikon, Switzerland) and was 18–19 mM. The alkalinity and background acetate concentration measured in control bottles did not change at the end of the experiment. The production of ^{14}C-labeled methane and CO_2 was measured every 1–3 h during the first day of incubation, and then daily during the next 2–3 days using a TriCarb 1600CA Scintillation Analyzer (Canberra Packard, Schlieren, CH) as described previously (Zepp-Falz *et al.*, 1999), but with minor modifications. Briefly, 1 mL of headspace was removed with a syringe, into which 1 mL of 2 M NaOH was drawn up. The syringe with the gas–NaOH mixture was shaken thoroughly to dissolve CO_2. Subsequently, the gas phase was injected into scintillation vials containing a toluene-based cocktail for the analysis of gaseous samples (Toluene Scintillator, Canberra Packard), while the liquid phase was transferred to scintillation vials containing a cocktail for liquid samples (Emulsifier Scintillator Plus, Canberra Packard). Isotopic experiments were conducted in five replicates. Methane formation rates was calculated from the observed linear increase in the concentration of labeled methane over time (Kotelnikova & Pedersen, 1998). The amount of $^{14}CH_4$ formed (C) were calculated as

$$\frac{D\ \mathrm{min}^{-1}\ \mathrm{sample}^{-1}}{\text{specific activity of the }^{14}\text{C substrate}}$$

where D is the disintegration. The total amount of methane formed in the radiotracer experiments was calculated as follows: The substrate concentrations that equaled the sum of the *in situ* pool of substrate and the amount of ^{14}C-labeled substrate added were divided by the ^{14}C-labeled substrate concentration, giving a coefficient denoted K, which shows the ratio of unlabeled to labeled substrate. The incorporation rate (μM methane h^{-1}) of labeled carbon into methane from 2-^{14}C-acetate or from $NaH^{14}CO_3$ was calculated as

$$\frac{K\ (C_2 - C_1)}{t_2 - t_1}$$

where C_1 and C_2 are the concentrations of radio-labeled methane at time-points t_1 and t_2. The SDs calculated for replicates and results from different sampling dates were in the range of 10–25%. The isotope effect of the heavy ^{14}C-carbon that was lower than 15% was not accounted for. The total rate of methane production was calculated as a sum of production from acetate and from bicarbonate, and the percentage-input from each substrate was calculated from this sum.

Results

Endogenous methanogenesis in nonamended sediment slurries

The rates of endogenous methane production of either aceto- or hydrogenotrophic methanogenesis in nonamended sediment slurries of Lake Baldegg enriched at different temperatures were measured using either ^{14}C-labeled acetate or ^{14}C-labeled bicarbonate. The resulting

© 2007 Federation of European Microbiological Societies
Published by Blackwell Publishing Ltd. All rights reserved

Table 1. The total rates of methanogenesis and rates and inputs derived from labeled acetate and bicarbonate in sediment slurries enriched at different temperatures

Enrichment and incubation temperature (°C)	Rate of methanogenesis measured by* (μM h^{-1})			Input (%) to total methane production from†	
	GC	^{14}C-bicarbonate	^{14}C-acetate	^{14}C-bicarbonate	^{14}C-acetate
5	0.32 ± 0.021	0.015 ± 0.003	0.35 ± 0.07	2–8	93–99
15	1.15 ± 0.082	0.16 ± 0.02	1.22 ± 0.2	8–13	87–96
30	3.75 ± 0.24	0.95 ± 0.11	3.2 ± 0.4	22–30	74–86
50	5.80 ± 0.55	5.65 ± 0.7	0.03 ± 0.005	92–99	1–6

*Data of one experiment with five replicates.
†Data of five independent experiments. The sum of hydrogenotrophic plus acetotrophic methanogenesis was assumed to be 100%.

methane production rates, measured during incubation of enriched slurries at the corresponding temperatures, were the highest in thermophilic enrichments and the lowest in the psychrophilic ones (Table 1). The ratios of the rates of acetotrophic and hydrogenothrophic methanogenesis differed considerably in the different slurries. The part of methane produced from acetate was the highest in psychrophilic enrichment and the lowest in the thermophilic one. It ranged from about 95% at 5 °C to < 5% at 50 °C (Table 1). The injection of H_2 into the head space of slurries incubated at 5 °C immediately increased the rate of methanogenesis two to three times in the presence of labeled bicarbonate (data not shown). In experiments with slurries enriched under thermophilic conditions, an intermediate production of labeled $^{14}CO_2$ from ^{14}C-acetate in the beginning of the experiment was observed (data not shown).

Methanogenesis and acetogenesis in sediment slurries amended with substrates

Methane and acetate production from H_2/CO_2 in fresh sediment slurries in a temperature range of 2–50 °C

The experiment was performed with slurries of fresh sediment samples that were incubated at eleven temperatures between 2 and 50 °C. The formation of acetate and methane from H_2/CO_2 available in high amounts (440/110 mmol L^{-1} slurry) by fresh sediment microbial communities was measured at all temperatures (Fig. 1). Acetogenesis prevailed on methanogenesis at 15 °C and below during the first month of incubation, and up to 50 mM acetate accumulated during this period. Acetate was also formed at a higher temperature, but it was subsequently consumed and methane was produced (Fig. 1). At temperatures below 15 °C, the accumulated acetate was not utilized even after 1 year of incubation. Accumulation of 50 mM acetate led to a slight acidification (pH 6.0), although the sediment slurries represented well-buffered systems.

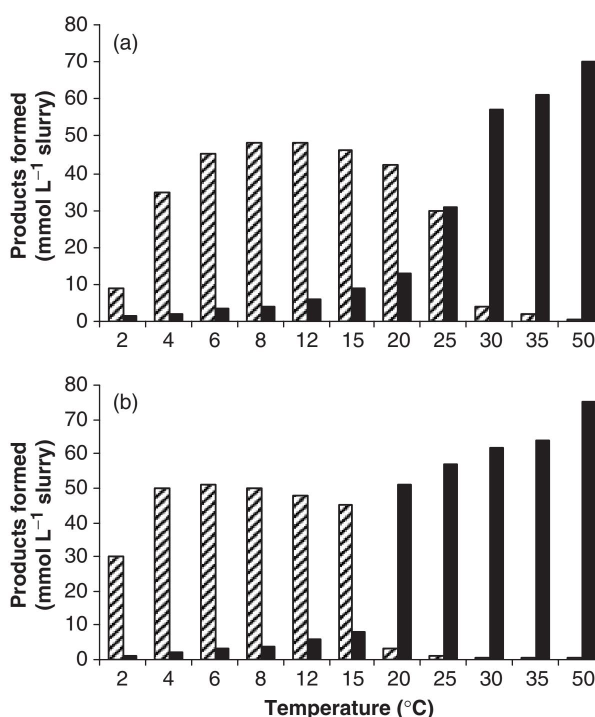

Fig. 1. Acetate (hatched bars) and methane (filled bars) produced from H_2/CO_2 by fresh sediment slurries after 1 month (a) and 2 months (b) of incubation in a temperature range of 2–50 °C.

Methane and acetate production from H_2/CO_2 in enriched sediment slurries in a temperature range of 5–70 °C

To study the rates and pathways of methanogenesis from H_2/CO_2 in a wide temperature range (5–70 °C), slurries of sediments were used where psychrophilic (5 °C), psychrotrophic (15 °C), mesophilic (30 °C), and thermophilic (50 °C) methanogenic microbial communities had been enriched previously. Experiments at 70 °C were performed with slurry enriched at 50 °C. The amount of substrate added was the same as in the experiment with fresh sediments (H_2:$CO_2 = 440:110$ mmol L^{-1} slurry). BES was

© 2007 Federation of European Microbiological Societies
Published by Blackwell Publishing Ltd. All rights reserved

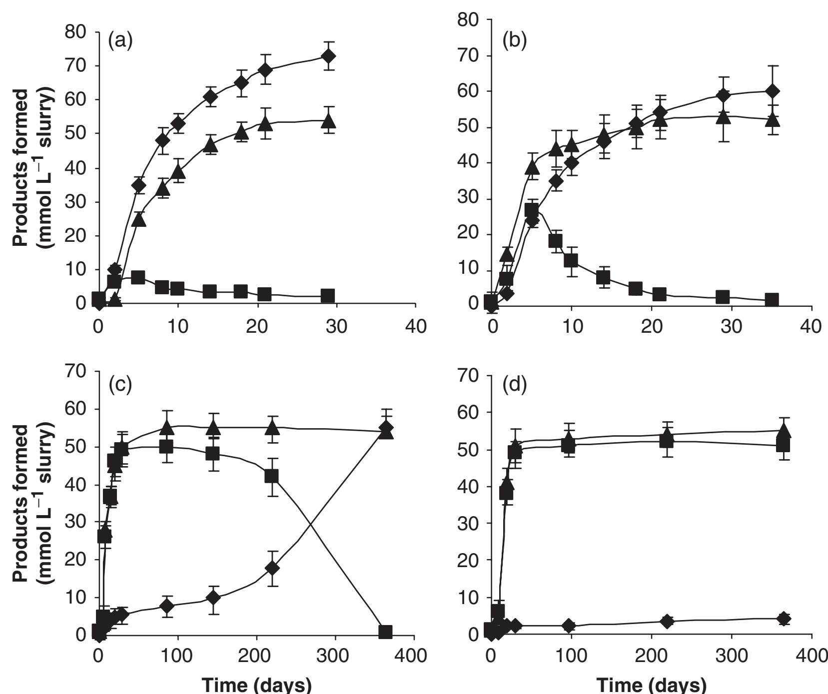

Fig. 2. Acetate and methane production from H_2/CO_2 by sediment slurries enriched and incubated at (a) 50 °C, (b) 30 °C, (c) 15 °C, and 5 °C (d). (♦) methane; (■) acetate in absence of BES; (▲) acetate in the presence of BES. BES, 2-bromoethane sulfonate.

added to inhibit methanogenesis with the aim to compare the rates of acetate production from H_2/CO_2 at different temperatures. No acetate formation from H_2 was detected at 70 °C; only methane was produced. About 5 mM acetate accumulated at 50 °C during the first 3 days of incubation. In the next week, acetate concentration decreased to 3 mM, and later to 1 mM and lower (Fig. 2a). Up to 30 mM acetate accumulated during the first 5 days of incubation in slurry enriched under mesophilic conditions. A large part of it was consumed with equimolar methane production in the subsequent few days (Fig. 2b). Acetate formed faster than methane in slurries enriched under psychrophilic and psychrotrophic conditions, and more than 50 mM of acetate accumulated. In contrast to the previous experiment with fresh sediments, slow methane production from acetate started at 15 °C after about 2 months, and acetate was slowly consumed during the subsequent 8 months of incubation until all acetate was consumed (Fig. 2c). Only small amounts of methane were produced in psychrophilic slurries and acetate was the predominant product of H_2/CO_2 consumption at 5 °C (Fig. 2d). During the periods of acetate accumulation, the rates of acetogenesis at low temperatures (5–15 °C) were similar with and without BES (Table 2), whereas the rates at 30 and 50 °C were higher with BES, indicating a possible methane production from H_2/CO_2 at these temperatures. The rates of methane production were lower than acetate production rates at 5–30 °C and higher at 50 °C (Table 2). Similar results were obtained when methanol was used as a substrate. At low temperatures, acetogenesis from methanol prevailed methanogenesis, and only at 50 °C was methane produced without intermediate production of acetate (data not shown).

Table 2. Rates of acetate and methane production from H_2/CO_2 before maximal accumulation of acetate by microbial communities enriched at different temperatures

Temperature (°C)	Rate of acetate production (mM day^{-1})		Rate of methane production (mM day^{-1})
	With BES	Without BES	
5	2.0	2.1	0.16
15	3.3	3.25	0.26
20	5.1	5.1	1.1
30	9	6	4.8
50	4	2.2	6.5

BES, 2-bromoethane sulfonate.

Influence of substrate concentration on methanogenesis

Influence of H_2/CO_2 concentration on aceto- and methanogenesis

Sediment slurries enriched at low temperatures were supplied with different amounts of H_2/CO_2 as a substrate and

© 2007 Federation of European Microbiological Societies
Published by Blackwell Publishing Ltd. All rights reserved

Table 3. The influence of initial H_2/CO_2 concentrations on acetate accumulation and consumption with methane formation in slurries enriched at low temperatures

			Period of acetate accumulation and consumption	
Temperature (°C)	Initial concentration of H_2/CO_2* (mM)	Maximum accumulated acetate concentration (mM)	Accumulation (days)	Consumption (lag phase) (months)
6	570/142	52	45	No consumption
	260/65	35	30	12 (2.5)
	200/50	15	20	4 (1)
15	570/142	46	20	5 (2)
	260/65	30	12	3 (1)
	200/50	12	8	1 (0)

*The concentrations given are nominal concentrations assuming that all H_2 and CO_2 of the gas phase, present at different partial pressures, would be dissolved in the aqueous phase.

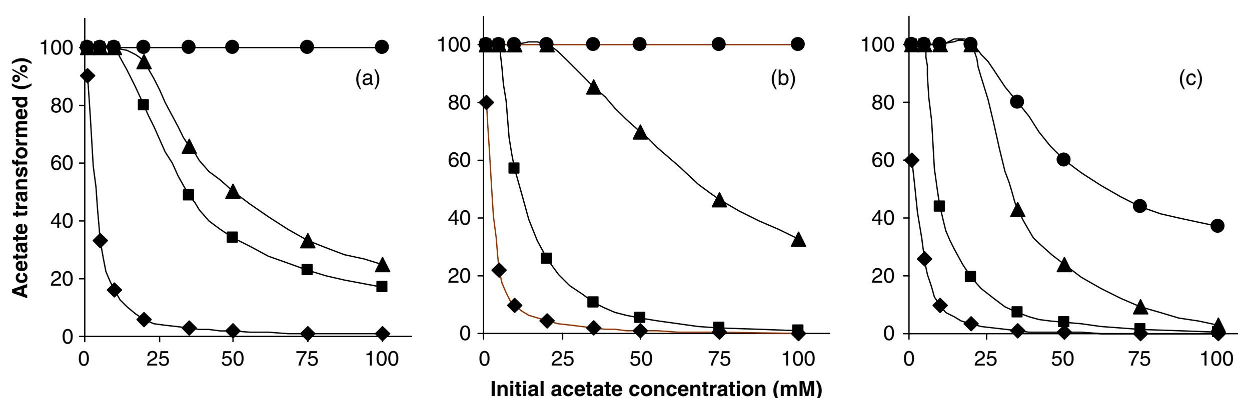

Fig. 3. Percentage of amended acetate transformed into methane after different periods of incubation at three different temperatures. (a) 30 °C: (♦) 6 days, (■) 18 days, (▲) 28 days, (●) 52 days; (b) 15 °C: (♦) 9 days, (■) 28 days, (▲) 74 days, (●) 120 days; (C) 5 °C: (♦) 28 days, (■) 74 days, (▲)120 days, and (●) 305 days.

acetate accumulation and consumption followed. Whereas acetate accumulated in all sediment slurry cultures, it was only in the culture incubated at 6 °C that acetate accumulated up to 52 mM and where it was not consumed during the subsequent months (Table 3). The general trend observed was that the lesser the acetate accumulated, the shorter the period was before methane formation started.

Influence of acetate concentration on the rate of methanogenesis

The influence of acetate concentrations on methane production at different temperatures was examined with enriched slurries. Sodium acetate was added at eight concentrations in a range between 1 and 100 mM to slurries preliminarily enriched at 5, 15, 30, and 50 °C. In the thermophilic enrichment, acetate was without a lag-phase transformed to methane within 4–5 days at all initial acetate concentrations. At 30 °C, the initial methane production rates were somewhat higher at lower acetate concentrations (1–20 mM). However, acetate was subsequently transformed into methane at the same rates at all concentrations, indicating pseudo-zero order kinetics in this concentration range (Fig. 3a). In psychrotrophic and psychrophilic enrichments, the initial rate of methanogenesis was strongly influenced by a higher acetate concentration. At acetate concentrations above 35 and 20 mM, methane production was inhibited at 15 and 5 °C, respectively, even after 1–3 months of incubation (Fig. 3b and c). Nevertheless, after these initial phases, methanogenesis proceeded with increasing rates at low temperatures, indicating a slow adaptation of psychrophilic communities to high acetate concentrations.

Discussion

The results obtained in this work confirmed the influence of temperature on the pathway of methanogenesis. The percentage of hydrogenotrophic methanogenesis was low at low temperatures and very high under thermophilic conditions. The increasing importance of hydrogenotrophic methanogenesis in total methane production with increasing

© 2007 Federation of European Microbiological Societies
Published by Blackwell Publishing Ltd. All rights reserved

temperatures has also been reported for slurries of sediments of Lake Constance (Chin & Conrad, 1995) and Lake Dagow (Glissmann *et al.*, 2004). The prevalence of acetotrophic vs. hydrogenotrophic methanogenesis at low temperature has been described for incubation experiments with samples from different cold environments. No conversion of $H^{14}CO_3^-$ into methane was found with sediment slurries of Lake Constance at a temperature below 20 °C, and methane production from ^{14}C-acetate was detected even at 2 °C (Schulz *et al.*, 1997). Peat incubation experiments conducted at 15 °C have indicated that $84 \pm 9\%$ of the methane was produced from acetate (Avery *et al.*, 1999) and in rice field soil, the contribution of hydrogenotrophic methanogenesis has been $13 \pm 5\%$ at 10 °C and about 25% at 15 °C (Fey & Conrad, 2000).

A detailed investigation of the temperature influence on the methanogenesis pathway and the structure of the microbial communities with rice field soil in a temperature range of 10–80 °C has shown that at a low temperature, acetotrophic prevailed in hydrogenotrophic methanogenesis and that at 50 °C, methane was produced exclusively from H_2/CO_2, while acetate was no longer consumed and accumulated (Fey & Conrad, 2000). The methanogenic communities in the rice field soil were found to be different at low, moderate, and high temperatures (Chin *et al.*, 1999). At a low temperature, *Methanosaetaceae* was conspicuous, and under mesophilic conditions *Methanosarcinaceae* dominated. A dramatic change in the archaeal community structure has been observed at temperatures above 37 °C, with the euarchaeotal rice cluster 1 becoming the dominating group (Fey *et al.*, 2001). Hence, the shift of the methanogenesis pathway might be a consequence of a shift in the archaeal community. However, there is still no clear mechanistic explanation on the prevalence of acetotrophic methanogenesis at low temperatures (Conrad, 1999).

In excess of H_2/CO_2, acetate was produced in slurries of fresh sediment of lake Baldegg during the first month of incubation at all the temperatures tested, except 50 °C. During the subsequent months, acetate was converted to methane at temperature above 15 °C; below this temperature, acetate was the final product of H_2/CO_2 consumption. This threshold of 15–20 °C has been observed before for low-temperature methanogenic environments like anoxic paddy soil, sediments of taiga pond, tundra permafrost soil, and lake sediments (Conrad *et al.*, 1989; Kotsyurbenko *et al.*, 1993, 1996; Zavarzin *et al.*, 1993; Nozhevnikova *et al.*, 1997).

High acetate concentrations inhibited initial methane production in sediment slurries up to 30 °C. However, after different lag-periods high acetate concentrations were also transformed into methane. A negative effect of high substrate concentration on the stability of methanogenesis has already been reported for a methanogenic pure culture (Westermann *et al.*, 1989) and a wastewater treatment system (Rebac *et al.*, 1995). Because anaerobic treatment of organic wastes at a low temperature has an interesting potential as an energy-saving technology, it is crucial to understand the response of the microbial community to conditions such as high substrate concentrations. The critical point in anaerobic reactor performance is an adaptation of the mesophilic microbial community to a low temperature as well as to high substrate concentrations (Lettinga *et al.*, 1999). Decrease of the rate of methanogenic activity due to the decrease of temperature may lead to an accumulation of volatile fatty acids, particularly acetate, butyrate, and propionate. Methane production in high initial acetate concentration sediment slurries after a lag-phase of several months indicated that the methanogenic microbial communities have the potential to adapt to these conditions even at a temperature as low as 5 °C. It would be interesting to further investigate whether this was due to an adaptation of acetoclastic methanogens that were already active in sediments before the experiment, or whether other acetate-utilizing methanogens started to develop and became predominant. In any case, the results obtained showed that the performance of the anaerobic community can be satisfactory even with a high substrate concentration and intermediate accumulation of volatile fatty acids if slow adaptation is allowed for.

References

Ammann AA & Rüttimann TB (1995) Simultaneous determination of small organic and inorganic anions in environmental water samples by ion-exchange chromatography. *J Chromatogr A* **706**: 259–269.

Avery GB Jr, Shannon RD, White JR, Martens CS & Alperin MJ (1999) Effect of seasonal changes in the pathways of methane production on the ^{13}C values of pore water methane in a Michigan peatland. *Global Biogeochem Cycles* **13**: 475–484.

Casper P, Chan OC, Furtado ALS & Adams DD (2003) Methane in an acidic bog lake: the influence of peat in the catchment on the biogeochemistry of methane. *Aquat Sci* **65**: 36–46.

Chin K-J & Conrad R (1995) Intermediary metabolism in methanogenic paddy soil and the influence of temperature. *FEMS Microbiol Ecol* **18**: 85–102.

Chin K-J, Lukow T & Conrad R (1999) Effect of temperature on structure and function of the methanogenic archaeal community in an anoxic rice field soil. *Appl Environ Microbiol* **65**: 2341–2349.

Conrad R (1999) Contribution of hydrogen to methane production and control of hydrogen concentrations in methanogenic soils and sediments. *FEMS Microbiol Ecol* **28**: 193–202.

Conrad R, Bak F, Seitz HJ, Thebrath B, Mayer HP & Schutz H (1989) Hydrogen turnover by psychrotrophic homoacetogenic and mesophilic methanogenic bacteria in anoxic paddy soil and lake sediment. *FEMS Microbiol Ecol* **6**: 285–294.

© 2007 Federation of European Microbiological Societies
Published by Blackwell Publishing Ltd. All rights reserved

Conrad R, Klose M & Claus P (2002) Pathway of CH_4 formation in anoxic rice field soil and rice roots determined by ^{13}C stable isotope fractionation. *Chemosphere* **47**: 797–806.
Fey A & Conrad R (2000) Effect of temperature on carbon and electron flow and on the archaeal community in methanogenic rice field soil. *Appl Environ Microbiol* **66**: 4790–4797.
Fey A & Conrad R (2003) Effect of temperature on the rate limiting step in the methanogenic degradation pathway in rice field soil. *Soil Biol Biochem* **35**: 1–8.
Fey A, Chin KJ & Conrad R (2001) Thermophilic methanogens in rice field soil. *Environ Microbiol* **3**: 295–303.
Fey A, Claus P & Conrad R (2004) Temporal change of ^{13}C isotope signatures and methanogenic pathways in rice field soil incubated anoxically at different temperatures. *Geochim Cosmochim Acta* **68**: 293–306.
Franzmann PD, Springer N, Ludwig W, Conway de Macario E & Rohde M (1992) A methanogenic Archaeon from Ace Lake, Antarctica: *Methanococcoides burtonii* sp. nov. *System Appl Microbiol* **15**: 573–581.
Franzmann PD, Liu YT, Balkwill DL, Aldrich HC, Conway de Macario E & Boone DR (1997) *Methanogenium frigidum* sp. nov., a psychrophilic, H_2-using methanogen from Ace Lake, Antarctica. *Int J Syst Bacteriol* **47**: 1068–1072.
Gächter R, Meyer JS & Mares A (1988) Contribution of bacteria to release and fixation of phosphorus in lake sediments. *Limnol Oceanogr* **33**: 1542–1558.
Glissmann K, Chin K-J, Casper P & Conrad R (2004) Methanogenic pathway and archaeal community structure in the sediment of eutrophic lake Dagow: effect of temperature. *Microbial Ecol* **48**: 389–399.
Jones JG & Simon BM (1985) Interaction of acetogens and methanogens in anaerobic freshwater sediments. *Appl Environ Microbiol* **49**: 944–948.
Jones JG, Simon BM & Gardener S (1982) Factors affecting methanogenesis and associated processes in the sediments of a stratified eutrophic lake. *J Gen Microbiol* **128**: 1–11.
Kelly CA & Chynoweth DP (1981) The contributions of temperature and of the input of organic matter in controlling rates of sediment methanogenesis. *Limnol Oceanogr* **26**: 891–897.
Kelts KU, Briegel K, Ghilardi K & Hsu K (1986) The limnogeology-ETH coring system. *Schweiz Z Hydrol* **1986**: 104–115.
Kotelnikova S & Pedersen K (1998) Distribution and activity of methanogens and homoacetogens in deep granitic aquifers at Äspo Hard Rock Laboratory, Sweden. *FEMS Microbiol Ecol* **26**: 121–134.
Kotsyurbenko OR, Nozhevnikova AN & Zavarzin GA (1993) Methanogenic degradation of organic matter by anaerobic bacteria at low temperature. *Chemosphere* **27**: 1745–1761.
Kotsyurbenko OR, Simankova MV, Nozhevnikova AN, Zhilina TN, Bolotina NP, Lysenko AM & Osipov GA (1995) New species of psychrophilic acetogens: *Acetobacterium bakii* sp. nov., *A. paludosum* sp. nov., *A. fimetarium* sp. nov. *Arch Microbiol* **163**: 29–34.
Kotsyurbenko OR, Nozhevnikova AN, Soloviova TI & Zavarzin GA (1996) Methanogenesis at low temperatures by microflora of tundra wetland soil. *Antonie van Levenhoek* **69**: 75–86.
Kotsyurbenko OR, Glagolev MV, Nozhevnikova AN & Conrad R (2001) Competition between homoacetogenic bacteria and methanogenic archaea for hydrogen at low temperature. *FEMS Microbiol Ecol* **38**: 153–159.
Kuivila KM, Murraym JW, Devol A-H & Novelli PC (1989) Methane production, sulfite reduction and competition for substrates in the sediments of Lake Washington. *Geochim Cosmochim Acta* **53**: 409–416.
Küsel K & Drake HL (1995) Effects of environmental parameters on the formation and turnover of acetate by forest soils. *Appl Environ Microbiol* **61**: 3667–3675.
Lettinga G, Rebac S, Parshina S, Nozhevnikova AN, Van Lier JB & Stams AJM (1999) High-rate anaerobic treatment of wastewater al low temperatures. *Appl Environ Microbiol* **65**: 1696–1702.
Lin CY, Noike T, Sato K & Matsumoto J (1987) Temperature characteristics of the methanogenesis process in anaerobic digestion. *Water Sci Technol* **19**: 299–310.
Lokshina LYa, Vavilin VA, Kettunen R, Rintala Ju, Holliger C, Rytov SV, Kotsyubenko OR & Nozhevnikova AN (2001) Evaluation of kinetic coefficients using integrated Monod and Haldane models for low-temperature acetoclastic methanogens. *Water Res* **35**: 2913–2922.
Lotter AF (1989) Evidence of annual layering in Holocene sediments of Soppensee, Switzerland. *Aquatic Sci* **51**: 19–30.
Murase J & Sugimoto A (2001) Spatial distribution of methane in the Lake Biwa sediments and its carbon isotopic compositions. *Geochem J* **35**: 257–263.
Namsaraev BB, Dulov LE, Sokolova EN & Zemskaya TI (1995) Bacterial methane production in the bottom sediments of Lake Baikal. *Microbiology* **64**: 346–352. (Translated from *Mikrobiologiya* **64**: 411–417).
Nozhevnikova AN, Holliger C, Ammann A & Zehnder AJB (1997) Methanogenesis in sediments from deep lakes at different temperatures (2–70 °C). *Water Sci Technol* **36**: 57–64.
Nozhevnikova AN, Zepp K, Vazquez F, Zahnder AJB & Holliger C (2003) Evidence for the existence of psychrophilic methanogenic communities in anoxic sediments of deep lakes. *Appl Environ Microbiol* **69**: 1832–1835.
Nüsslein B & Conrad R (2000) Methane production in eutrophic Lake Plussee: seasonal change, temperature effect and metabolic processes in the produndal sediment. *Arch Hydrobiol* **149**: 597–623.
Nüsslein B, Chin KJ, Eckert W & Conrad R (2001) Evidence for anaerobic syntrophic acetate oxidation during methane production in the profundal sediment of subtropical Lake Kinneret (Israel). *Environ Microbiol* **3**: 460–470.
Rebac S, Ruskova J, Gerbens S, van Lier JB, Stams AJM & Lettinga G (1995) High-rate anaerobic treatment of wastewater under psychrophilic conditions. *J Ferment Bioeng* **80**: 499–506.

© 2007 Federation of European Microbiological Societies
Published by Blackwell Publishing Ltd. All rights reserved

Schulz S & Conrad R (1996) Influence of temperature on pathways to methane production in the permanently cold profundal sediment of Lake Constance. *FEMS Microbiol Ecol* **20**: 1–14.

Schulz S, Hidetoshi M & Conrad R (1997) Temperature dependence of methane production from different precursors in a profundal sediment (Lake Constance). *FEMS Microbiol Ecol* **22**: 207–213.

Simankova MV, Kotsyurbenko OR, Stackebrandt E, Kostrikina NA, Lysenko AM, Osipov GA & Nozhevnikova AN (2000) *Acetobacterium tundrae* sp. nov., a new psychrophilic acetogenic bacterium from tundra soil. *Arch Microbiol* **174**: 440–447.

Simankova MV, Parshina SN, Tourova TP, Kolganova TV, Zehnder AJB & Nozhevnikova AN (2001) *Methanosarcina lacustris* sp. nov., a new psychrotolerant methanogenic archaeon from anoxic lake sediments. *Syst Appl Microbiol* **24**: 62–367.

Simankova MV, Kotsyurbenko OR, Lueders T, Nozhevnikova AN, Wagner B, Conrad R & Friedrich MW (2003) Isolation and characterization of new strains of methanogens from cold terrestrial habitats. *Syst Appl Microbiol* **26**: 312–318.

Thebrath B, Rothfuss F, Whiticar MJ & Conrad R (1993) Methane production in littoral sediment of Lake Constance. *FEMS Microbiol Ecol* **102**: 279–289.

Westermann P, Ahring BK & Mah RA (1989) Temperature compensation in *Methanosarcina barkeri* by modulation of hydrogen and acetate affinity. *Appl Environ Microbiol* **55**: 1262–1266.

Zavarzin GA, Kotsyrbenko OR, Soloviova TI & Nozhevnikova AN (1993) The temperature threshold in the development of methanogenic versus acetogenic microbial community from the tundra soil. *Doklady Russ Acad Sci* **329**: 116–118 (in Russian).

Zehnder AJB & Brock TD (1980) Anaerobic methane oxidation: occurrence and ecology. *Appl Environ Microbiol* **31**: 99–107.

Zehnder AJB, Huser B & Brock TD (1979) Measuring radioactive methane with the liquid scintillation counter. *Appl Environ Microbiol* **37**: 897–899.

Zeikus JG & Winfrey MR (1976) Temperature limitation of methanogenesis in aquatic sediments. *Appl Environ Microbiol* **31**: 9–107.

Zepp-Falz K, Holliger C, Grosskopf R, Liesack W, Nozhevnikova AN, Muller B, Wehrli B & Hahn D (1999) Vertical distribution of methanogens in the anoxic sediment of Rotsee (Switzerland). *Appl Environ Microbiol* **65**: 2402–2408.

© 2007 Federation of European Microbiological Societies
Published by Blackwell Publishing Ltd. All rights reserved