

## Stable isotope biogeochemistry of methane formation in profundal sediments of Lake Kinneret (Israel)

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### *Abstract*

Methane production in profundal sediments of Lake Kinneret was recently found to be, to a large extent, syntrophically coupled to the oxidation of acetate, an apparently unique feature of CH<sub>4</sub> biogeochemistry in lake sediments. Our measurements of the conversion of <sup>14</sup>CO<sub>2</sub> and of [2-<sup>14</sup>C]acetate to CH<sub>4</sub> at both 15 and 30°C were in agreement with this observation. Measurement of the fraction of <sup>14</sup>CO<sub>2</sub>-derived CH<sub>4</sub> together with δ<sup>13</sup>CH<sub>4</sub> and δ<sup>13</sup>CO<sub>2</sub> allowed the calculation of δ<sup>13</sup>CH<sub>4</sub> values originating from either CO<sub>2</sub> (δ<sub>mc</sub>) or acetate (δ<sub>ma</sub>). Assuming α-values for <sup>13</sup>C fractionation during CO<sub>2</sub> reduction to CH<sub>4</sub> of 1.06–1.07, CH<sub>4</sub> production from acetate required a relatively large fractionation factor that increased with depth and resulted in a δ<sub>ma</sub> from –50 to –60‰. This result is consistent with syntrophic acetate conversion to CH<sub>4</sub> rather than acetoclastic methanogenesis. The apparent fractionation factor (α) between CO<sub>2</sub> and CH<sub>4</sub> increased with depth from 1.045 to 1.065. The δ<sup>13</sup>C of sedimentary particulate organic matter increased with depth from –28 to –24‰. Incubation of sediment samples from layers in the upper 5 cm resulted in a steady increase of α, both at 15 and 30°C. Unlike deeper sediment layers, it remained constant, with δ<sup>13</sup>CH<sub>4</sub> decreasing with incubation time. This behavior is indicative of sediments in which methanogenesis is extremely limited by substrate. Collectively, our experiments demonstrate the usefulness of <sup>13</sup>C data to elucidate the pathway of CH<sub>4</sub> production and confirm the unique biogeochemical features of CH<sub>4</sub> biogeochemistry in Lake Kinneret.

Methane production is one of the key terminal processes in anaerobic decomposition of organic matter in sediments (Capone and Kiene 1988). Below the upper 1–2 mm, profundal sediments of stratified eutrophic lakes are permanently anoxic. Because sulfate is usually limiting in freshwater lakes, methanogenic degradation of organic matter becomes the dominant process. In North American lakes, for example, more than half of the total carbon input to the sediment was found to be converted to CH<sub>4</sub> (Rudd and Hamilton 1978). The immediate substrates for CH<sub>4</sub> production are acetate and H<sub>2</sub>/CO<sub>2</sub>, which contribute to various extents (Conrad 1999). Theoretically, a ratio of 2:1 or higher is expected (Conrad 1999). The CH<sub>4</sub> production processes are influenced by the conditions in the sediment, notably by interaction with sulfate reduction (Winfrey and Zeikus 1979; Lovley and Klug 1983; Bak and Pfennig 1991), supply of organic substrate (Schulz and Conrad 1995), and temperature

(Zeikus and Winfrey 1976; Schulz and Conrad 1996; Schulz et al. 1997).

The processes involved in CH<sub>4</sub> production also influence the biogeochemistry of stable isotopes (Whiticar 1999). Methanogenesis from H<sub>2</sub>/CO<sub>2</sub> results in a larger fractionation against <sup>13</sup>C and, thus, in lower δ<sup>13</sup>CH<sub>4</sub> values than methanogenesis from acetate (Games et al. 1978; Krzycki et al. 1987; Gelwicks et al. 1994). The isotopic signature of the methanogenic precursors also influences the <sup>13</sup>C isotopic signature of the produced CH<sub>4</sub>. Acetate, for example, seems to have a different <sup>13</sup>C isotopic signature when it is produced by homoacetogenic CO<sub>2</sub> reduction rather than by fermentation of organic matter (Blair et al. 1985; Gelwicks et al. 1989). The isotopic signature of CO<sub>2</sub> depends on whether the pool is generated mainly from dissociated carbonate or respired organic matter. Carbon source, substrate limitation and prevalent biogeochemical pathways all influence the stable isotope composition of CH<sub>4</sub> in aquatic sediments (Hornibrook et al. 2000).

Methane biogeochemistry has mainly been studied in sediments of temperate lakes, whereas subtropical lakes such as Lake Kinneret have not received much attention. Lake Kinneret is a warm monomictic lake located in Galilea (Israel). The lake has a surface of 170 km<sup>2</sup> and a volume of 4 × 10<sup>9</sup> m<sup>3</sup>, is stratified during April–November, and exhibits at the central lake station a constant sediment temperature of 15°C, with about 1°C interannual variation. Information on dynam-

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ics of nutrients and carbon are found in Serruya (1971) and Serruya et al. (1980). Sulfate reduction has already been studied in Lake Kinneret (Eckert and Trüper 1993; Hadas and Pinkas 1995). Recently, we found that much of the CH<sub>4</sub> production in Lake Kinneret sediment seems to occur via syntrophic acetate degradation (Nüsslein et al. 2001). In this pathway, acetate is first oxidized to 4H<sub>2</sub> + 2CO<sub>2</sub>, which are then converted by hydrogenotrophic methanogenic archaea to CH<sub>4</sub> + CO<sub>2</sub>.

We hypothesize that the operation of such a pathway should also affect the stable isotope biogeochemistry of CH<sub>4</sub>. An additional feature possibly affecting the isotope biogeochemistry of CH<sub>4</sub> in Lake Kinneret sediment is the abundance of calcite, which affects the chemistry of available CO<sub>2</sub> (Serruya 1971; Stiller and Magaritz 1974). Therefore, we measured vertical profiles of δ<sup>13</sup>CH<sub>4</sub> and δ<sup>13</sup>CO<sub>2</sub> in Lake Kinneret sediment and studied the change of the isotopic composition on incubation at two (in situ and elevated) temperatures. The interpretation of the stable isotopic signatures was constrained by simultaneous measurement of <sup>14</sup>CO<sub>2</sub> conversion to <sup>14</sup>CH<sub>4</sub>.

## Materials and methods

**Sampling**—Sediment samples from Lake Kinneret were taken as described by Nüsslein et al. (2001). In June 1999 and November 2000, five parallel sediment cores were sampled from the central lake station using a gravity corer (Tessenow et al. 1977). In the laboratory, the cores were dissected under an argon atmosphere. The upper 5 cm of the cores were sliced into layers of 1-cm thickness; the sediment from 5 to 20 cm depth was sectioned into 3-cm layers. The sediment sections were transferred into gas-tight bottles and kept under an argon atmosphere.

**Sediment incubations and rate measurements**—Rates of CH<sub>4</sub> production, turnover of [2-<sup>14</sup>C]acetate, and conversion of NaH<sup>14</sup>CO<sub>3</sub> into <sup>14</sup>CH<sub>4</sub> bicarbonate were determined as described by Nüsslein et al. (2001). For these measurements, the sediment sections were stored at 4°C and transported to Germany and kept at 4°C until processing (0.5–6 months). Methane production rates remained the same as those measured in a control immediately in the lakeside laboratory. All rates were measured in triplicate incubations at in situ (15°C) and elevated (30°C) temperatures. The respiratory index (RI) for the degradation of [2-<sup>14</sup>C]acetate was determined at the end of the experiment with Eq. 1 after the addition of 1 ml of 1 mol L<sup>-1</sup> H<sub>2</sub>SO<sub>4</sub> to remove all residual CO<sub>2</sub>.

$$RI = {}^{14}\text{CO}_2 / ({}^{14}\text{CO}_2 + {}^{14}\text{CH}_4) \quad (1)$$

The fraction of CH<sub>4</sub> produced from H<sub>2</sub>/CO<sub>2</sub> (f<sub>H<sub>2</sub></sub>) was determined from the conversion of H<sup>14</sup>CO<sub>3</sub> to <sup>14</sup>CH<sub>4</sub> using the specific radioactivity of CH<sub>4</sub> (SR<sub>CH<sub>4</sub></sub>) and CO<sub>2</sub> (SR<sub>CO<sub>2</sub></sub>) (Conrad et al. 1989).

$$f_{\text{H}_2} = \text{SR}_{\text{CH}_4} / \text{SR}_{\text{CO}_2} \quad (2)$$

The turnover time (τ) of [2-<sup>14</sup>C]acetate was calculated from the logarithmic transformation with time into <sup>14</sup>CH<sub>4</sub> plus <sup>14</sup>CO<sub>2</sub> (Phelps and Zeikus 1984). We assumed that the max-

imum value of <sup>14</sup>CH<sub>4</sub> and <sup>14</sup>CO<sub>2</sub> produced was equivalent to 100% of the acetate available to microorganisms, giving Eq. 3.

$$\tau = t / \ln[1 - ({}^{14}\text{CO}_2 + {}^{14}\text{CH}_4)_t / ({}^{14}\text{CO}_2 + {}^{14}\text{CH}_4)_{\text{max}}] \quad (3)$$

**Stable isotope analysis**—During the incubations for measurement of CH<sub>4</sub> production, gas samples (0.25–1.0 ml) were also analyzed for δ<sup>13</sup>CH<sub>4</sub> and δ<sup>13</sup>CO<sub>2</sub> using a gas chromatograph combustion–isotope ratio mass spectrometer (GCC-IRMS; Thermoquest, purchased from Finnigan) as described in detail by Conrad et al. (2002).

The dissolved gases in the pore water were extracted immediately during the dissection procedure. After transfer of the core sections into gas-tight bottles with argon headspace and heavy shaking for 60 s, gas samples were taken and injected into stoppered glass test tubes completely filled with saturated NaCl solution. The injection was done so that the gas sample replaced the NaCl solution and formed a gas bubble. To prevent any gas loss, the tubes were stored upside down.

Total sediment carbon (C<sub>tot</sub>) was analyzed after air drying sediment aliquots at room temperature. Particulate sedimentary organic (C<sub>org</sub>) and inorganic carbon were separated by dissolving the latter with aliquots of HCl until bubble formation of CO<sub>2</sub> had ceased, followed by air drying at room temperature. The analysis of these δ<sup>13</sup>C sediment samples was carried out at the Institute for Soil Science and Forest Nutrition (IBW) at the University of Göttingen, Germany (courtesy of Heinz Flessa).

**Calculations related to stable isotope fractionation**—The fractionation factor (α) for the reaction 4H<sub>2</sub> + CO<sub>2</sub> → CH<sub>4</sub> + 2H<sub>2</sub>O was defined by Eq. 4.

$$\alpha = (\delta^{13}\text{CO}_2 + 1,000) / (\delta\text{mc} + 1,000) \quad (4)$$

Values of δmc (i.e., δ<sup>13</sup>CH<sub>4</sub> produced from the reduction of CO<sub>2</sub>) were calculated from measured values of δ<sup>13</sup>CO<sub>2</sub> using Eq. 4. Values of δma (i.e., δ<sup>13</sup>CH<sub>4</sub> produced from cleavage of acetate: CH<sub>3</sub>COOH → CH<sub>4</sub> + CO<sub>2</sub>) were calculated from the mass balance in Eq. 5.

$$\delta^{13}\text{CH}_4 = f_{\text{H}_2}\delta\text{mc} + (1 - f_{\text{H}_2})\delta\text{ma} \quad (5)$$

Radiotracer experiments determined f<sub>H<sub>2</sub></sub> (Eq. 2).

## Results and discussion

**Methane production and turnover of <sup>14</sup>CO<sub>2</sub> and [2-<sup>14</sup>C]acetate**—Methane production in sediment samples was studied during the stratification period of the lake (April–November). During this period, sulfide and CH<sub>4</sub> continuously accumulate in the hypolimnion (Eckert and Trüper 1993; Hadas and Pinkas 1995). Vertical profiles of CH<sub>4</sub> production rates in the sediment, measured at in situ (15°C) and elevated (30°C) temperatures, are shown in Fig. 1A. Rates of CH<sub>4</sub> production were similar in the cores taken in June and in November, demonstrating reproducible conditions over the year. However, the rates were consistently higher at 30 than at 15°C. This result is intriguing, since microorganisms in the sediment experience a rather constant temperature of

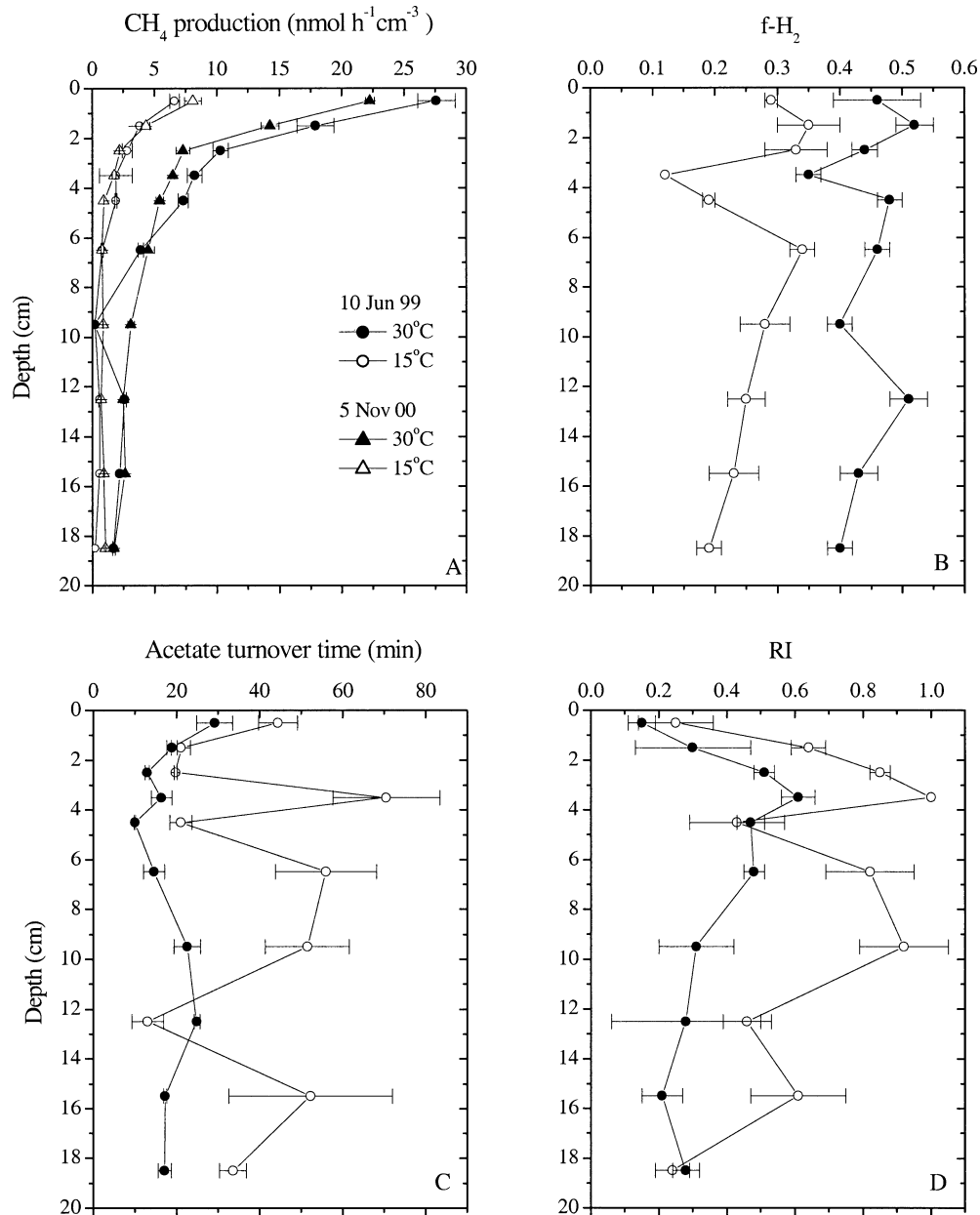


Fig. 1. Vertical profiles of (A)  $\text{CH}_4$  production rates, (B) fractions of  $\text{CH}_4$  produced from  $^{14}\text{CO}_2$ , (C) turnover times of  $[2-^{14}\text{C}]$ acetate, and (D) respiratory indices of  $[2-^{14}\text{C}]$ acetate oxidation, measured in sections of sediment cores taken in June 1999 and incubated. Rates of  $\text{CH}_4$  production were also measured in sediment cores taken in November 2000. The data are means  $\pm$  SE of triplicate incubations.

$15^\circ\text{C}$  year-round. Nevertheless, the optimum temperature of microbial  $\text{CH}_4$  production seems to be higher. This has been observed in numerous studies before (e.g., Zeikus and Winfrey 1976; Schulz et al. 1997), not only for  $\text{CH}_4$  production, but also for other processes (e.g., Yayanos 1986; Isaksen and Joergensen 1996).

Sediment cores sampled in June 1999 were used to determine the fraction ( $f_{\text{H}_2}$ ) of  $\text{CH}_4$  produced from  $\text{H}_2/\text{CO}_2$  by measuring the conversion of  $^{14}\text{CO}_2$  to  $^{14}\text{CH}_4$  and the acetate turnover by measuring the conversion of  $[2-^{14}\text{C}]$ acetate to  $^{14}\text{CH}_4$  plus  $^{14}\text{CO}_2$  (Fig. 1B–D). The data obtained at  $15^\circ\text{C}$

were more scattered than at  $30^\circ\text{C}$ , probably because of the low  $\text{CH}_4$  production rates. Nevertheless, the values of  $f_{\text{H}_2}$  were consistently higher at  $30^\circ\text{C}$  than at  $15^\circ\text{C}$  and were also higher than the theoretical value of 0.33 (Conrad 1999). The turnover of acetate also was more rapid at  $30^\circ\text{C}$  than at  $15^\circ\text{C}$  (Fig. 1C). A relatively large fraction of the acetate-methyl carbon was oxidized to  $\text{CO}_2$  rather than reduced to  $\text{CH}_4$ —more so at  $15^\circ\text{C}$  than at  $30^\circ\text{C}$ , as indicated by the RI (Fig. 1D). Nevertheless, conversion rates of acetate to  $\text{CH}_4$  were sufficient to explain the rates of total  $\text{CH}_4$  production (data not shown). Collectively, these results confirm our previous con-

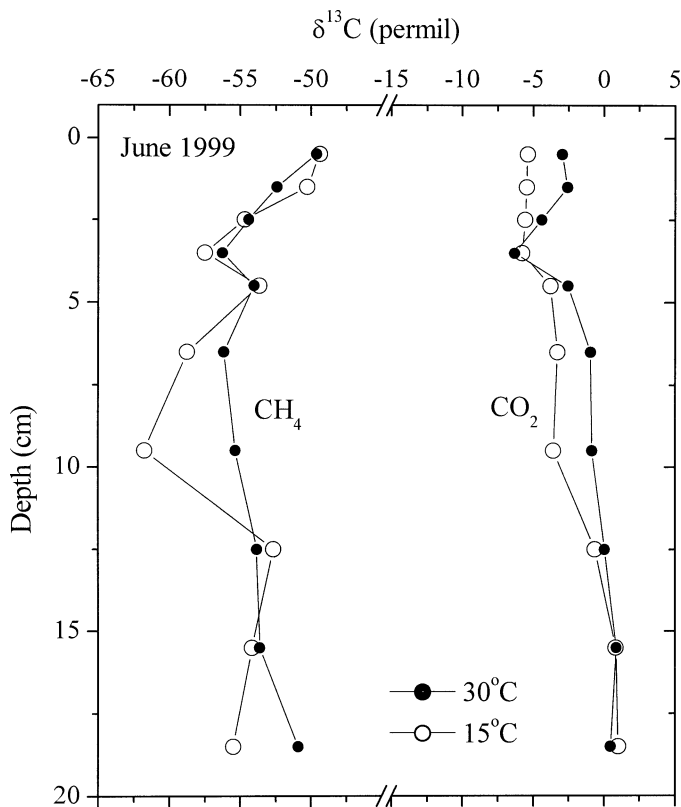


Fig. 2. Vertical profile of  $\delta^{13}\text{CH}_4$  and  $\delta^{13}\text{CO}_2$  produced in sediment sections sampled in June 1999 after 196 and 144 h incubation at in situ ( $15^\circ\text{C}$ ) and elevated ( $30^\circ\text{C}$ ) temperatures, respectively.

clusion of syntrophic acetate oxidation coupled to  $\text{H}_2/\text{CO}_2$ -dependent methanogenesis (Nüsslein et al. 2001). Whereas  $f_{\text{H}_2}$  and acetate turnover were fairly constant with depth (Fig. 1B,C), the RI reached a maximum at about 3–10 cm depth, depending on temperature, and then decreased slightly further on (Fig. 1D). Respiratory processes such as sulfate reduction could not explain the relatively high RI values (Nüsslein et al. 2001). Possibly, syntrophic acetate conversion to  $\text{CH}_4$  has reached a maximum at these depths.

**Stable isotopic signature of  $\text{CH}_4$  produced from  $\text{CO}_2$  and acetate during incubation**—For comparison, the samples of the same sediment cores were used to measure the  $\delta^{13}\text{C}$  of the  $\text{CH}_4$  and  $\text{CO}_2$  released after incubation at  $15^\circ\text{C}$  (200 h) and  $30^\circ\text{C}$  (150 h) (Fig. 2). These data, together with the values of  $f_{\text{H}_2}$  (Fig. 1B), were then used to calculate the isotopic signature of the  $\text{CH}_4$  produced from  $\text{CO}_2$  ( $\delta\text{mc}$ ) versus acetate ( $\delta\text{ma}$ ) (Fig. 3). The calculation assumes a fractionation factor for the conversion of  $\text{CO}_2$  to  $\text{CH}_4$  ( $\alpha_{\text{CO}_2}$ ) of either 1.06 (Fig. 3A) or 1.07 (Fig. 3B). According to literature data (Whiticar 1999; Hornibrook et al. 2000), these values of  $\alpha_{\text{CO}_2}$  represent the most likely range. The different temperatures ( $15$  vs.  $30^\circ\text{C}$ ) should have only a marginal influence ( $\pm 0.004$ ) on the magnitude of  $\alpha_{\text{CO}_2}$  (Blair et al. 1993). Depending on  $\alpha_{\text{CO}_2}$ , values of  $\delta\text{mc}$  were around  $-55$  to  $-62\text{‰}$  ( $\alpha_{\text{CO}_2} = 1.06$ ) or  $-65$  to  $-71\text{‰}$  ( $\alpha_{\text{CO}_2} = 1.07$ ). Values increased slightly with depth and were slightly more negative at  $15$  versus  $30^\circ\text{C}$ .

Values of  $\delta\text{ma}$ , on the other hand, showed more pronounced differences with lower values at  $15$  versus  $30^\circ\text{C}$ . In addition, values of  $\delta\text{ma}$  showed a minimum at 4–10 cm depth (Fig. 3)—that is, at the same range where the RI maximum was seen (Fig. 1D)—irrespective of the  $\alpha_{\text{CO}_2}$  and temperature. The minimum values of  $\delta\text{ma}$  were  $-55$  to  $-62\text{‰}$  for  $\alpha_{\text{CO}_2} = 1.06$  and  $-48$  to  $-59\text{‰}$  for  $\alpha_{\text{CO}_2} = 1.07$ . The highest values were around  $-41$  to  $-44\text{‰}$  for  $\alpha_{\text{CO}_2} = 1.06$  and  $-34$  to  $-40\text{‰}$  for  $\alpha_{\text{CO}_2} = 1.07$ . Similar values of  $\delta\text{ma}$  were obtained when  $f_{\text{H}_2}$  was assumed to be constant (Fig. 3B), indicating that the shape of the vertical profile of  $\delta\text{ma}$  was mainly caused by the measured values of  $\delta^{13}\text{CO}_2$  and  $\delta^{13}\text{CH}_4$ . Hence, values of  $\delta\text{ma}$  were low compared to literature data on other methanogenic wetlands (between  $-30$  and  $-45\text{‰}$ , Sugimoto and Wada 1993; Avery et al. 1999). Relatively low values of  $\delta\text{ma}$  ( $< -50$  to  $-60\text{‰}$ ) also were obtained in incubation experiments with sediment cores taken in November 2000 (see below).

**Stable isotopic signature of  $\text{CH}_4$  and  $\text{CO}_2$  and implications for the  $\text{CH}_4$  production process**—Vertical profiles of  $\delta^{13}\text{C}$  in  $\text{CH}_4$ ,  $\text{CO}_2$ , and sedimentary carbon were measured in the cores obtained in November 2000 (Fig. 4). Values of  $\delta^{13}\text{CO}_2$  increased with depth from  $-23\text{‰}$  at the surface ( $-23\text{‰}$  in the water overlying the sediment) to about  $0\text{‰}$  at  $>12$  cm depth (Fig. 4). The  $\text{CO}_2$  pool of Lake Kinneret sediment was apparently influenced by the sedimentary carbonates.  $\text{CaCO}_3$  ( $-3$  to  $-1\text{‰}$ ) and interstitial carbonates ( $-5$  to  $+11\text{‰}$ ) are reported to be relatively heavy in  $^{13}\text{C}$  (Stiller and Magaritz 1974).  $\delta^{13}\text{C}$  of organic carbon increased with depth from  $-28$  to  $-24\text{‰}$  (Fig. 4). Total sedimentary carbon (including carbonate) also increased, but from  $-17$  to  $-10\text{‰}$  (Fig. 4). Although the dissolved  $\text{CO}_2$  was depleted in  $^{13}\text{C}$  compared to total sedimentary carbon in the surface sediment, it was more  $^{13}\text{C}$ -enriched at depths  $>3$  cm (Fig. 4). This observation indicates that the  $\text{CO}_2$  pool was progressively (with depth) dominated by the relatively heavy carbonate-derived  $\text{CO}_2$  versus the relatively light organic carbon-derived  $\text{CO}_2$ , probably because the rates of microbial mineralization of organic carbon decreased with depth.

Values of  $\delta^{13}\text{CH}_4$  slightly decreased from  $-60\text{‰}$  at the surface to  $-63\text{‰}$  at  $>10$  cm depth (Fig. 5), so that the apparent fractionation factor between the measured  $\delta^{13}\text{CO}_2$  and  $\delta^{13}\text{CH}_4$  also increased (Fig. 5). Such a distribution of  $\delta^{13}\text{C}$ , classified as Type I distribution by Hornibrook et al. (2000), is characteristic for a progressive prevalence with depth of methanogenesis via  $\text{CO}_2$  reduction relative to acetoclastic methanogenesis. Slopes of Type I distributions seem to be less steep if the transfer of labile organic carbon to the methanogenic zone is diminished, consequently resulting in a relatively larger contribution of  $\text{CO}_2$ -dependent (larger fractionation) compared to acetoclastic (smaller fractionation) methanogenesis (Hornibrook et al. 2000). In Lake Kinneret sediment, the slope was extremely small, suggesting that not only the relative quantity but also the quality of  $\text{CO}_2$  reduction changed with increasing depth. We suggest that this particular feature might be caused by methanogenesis following syntrophic acetate oxidation, since this pathway would presumably result in a larger fractionation of  $^{13}\text{C}$  than either acetoclastic or  $\text{CO}_2$ -dependent methanogenesis

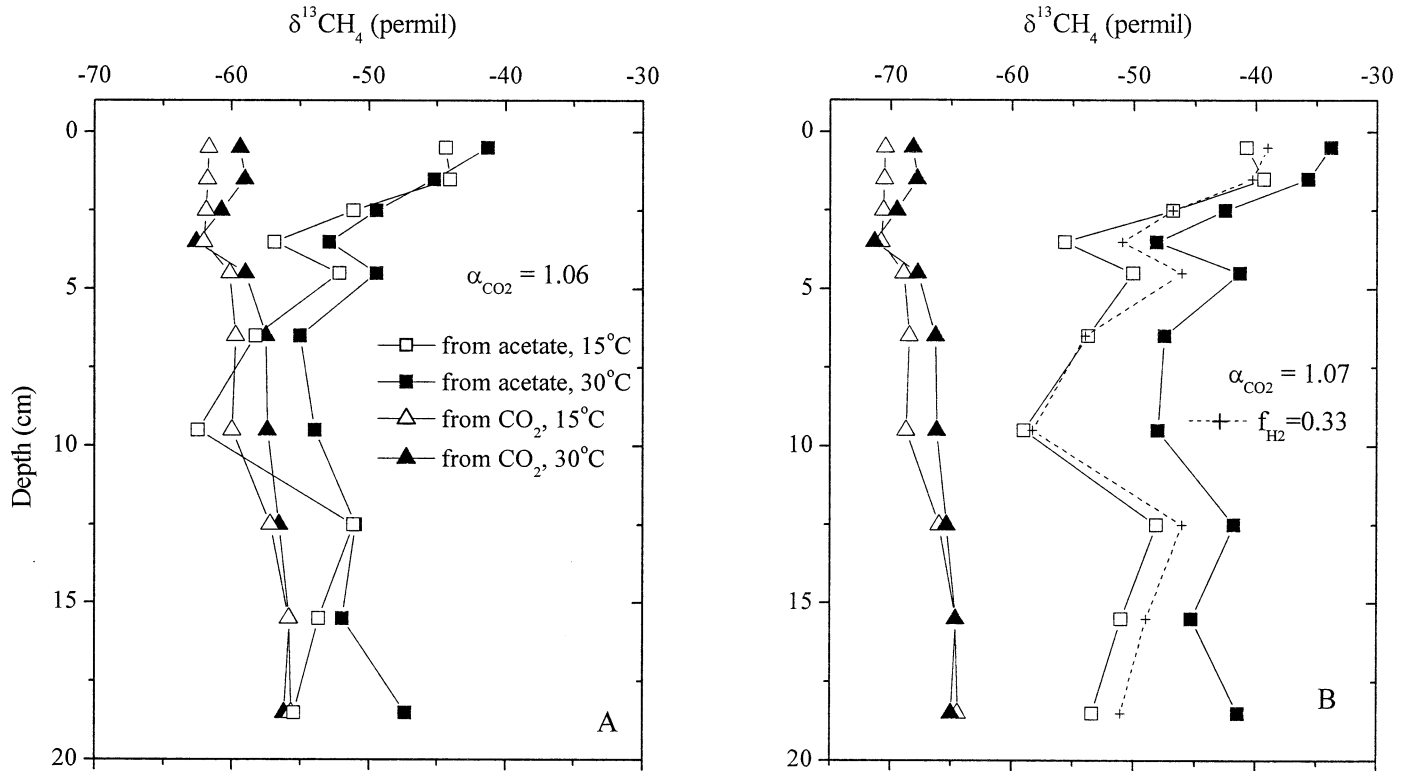


Fig. 3. Vertical profile of  $\delta\text{mC}$  (i.e.,  $\delta^{13}\text{C}$  of  $\text{CH}_4$  produced from  $\text{CO}_2$ ) and  $\delta\text{mA}$  (i.e.,  $\delta^{13}\text{C}$  of  $\text{CH}_4$  produced from acetate), calculated from the values of  $\delta^{13}\text{C}_{\text{CH}_4}$  and  $\delta^{13}\text{C}_{\text{CO}_2}$  from Fig. 2 and the values of  $f_{\text{H}_2}$  from Fig. 1, using Eq. 5 and assuming (A)  $\alpha_{\text{CO}_2} = 1.06$  and (B)  $\alpha_{\text{CO}_2} = 1.07$ .

alone (see below). A particularly strong fractionation is required, since the input of heavy  $\text{CO}_2$  from the carbonate pool, which should actually cause a steeper distribution, must be counterbalanced.

When sediment samples from different depths were incubated under anaerobic conditions, the values of  $\delta^{13}\text{C}_{\text{CH}_4}$  and  $\delta^{13}\text{C}_{\text{CO}_2}$  progressed in a way comparable to a Type I distribution (i.e., the apparent fractionation factor between  $\text{CO}_2$  and  $\text{CH}_4$  became progressively larger both at 15 and 30°C; Fig. 6). Both the initial values and the progression during incubation showed the Type I distribution. However, there was a systematic difference in the progressive change with incubation time when sediment samples from the upper (0–5 cm) and the lower (>5 cm) sediment layers were compared (Fig. 6). In the upper sediment,  $\delta^{13}\text{C}_{\text{CH}_4}$  stayed constant with time while  $\delta^{13}\text{C}_{\text{CO}_2}$  increased. In the lower sediment, on the other hand,  $\delta^{13}\text{C}_{\text{CH}_4}$  decreased while  $\delta^{13}\text{C}_{\text{CO}_2}$  stayed constant (30°C; Fig. 6B) or also decreased (15°C; Fig. 6A). The opposite behavior, simultaneous increase with depth of both  $\delta^{13}\text{C}_{\text{CH}_4}$  and  $\delta^{13}\text{C}_{\text{CO}_2}$  has been classified as Type II distribution. Type II distribution, typically found in marine sediment, is regarded to be caused by extreme substrate limitation of methanogenesis (Hornibrook et al. 2000). Extreme substrate limitation probably occurs in the lower sediment layers of Lake Kinneret, where rates of  $\text{CH}_4$  production are low. Nevertheless, low values of  $\delta\text{mA}$  (<–50 to –60‰) were obtained when the  $\delta^{13}\text{C}_{\text{CO}_2}$  and  $\delta^{13}\text{C}_{\text{CH}_4}$  data observed in the incubation experiments with sediment from >4 cm depth (Fig. 6) were used in mass balance calculations (Eq. 5), as-

suming  $f_{\text{H}_2} = 0.33$  and  $\alpha_{\text{CO}_2} = 1.07$ . These  $\delta\text{mA}$  values were as low as those observed in the sediment taken in June 1999 (Fig. 3), indicating that substantial  $^{13}\text{C}$  fractionation occurred during methanogenesis despite the generally low rates of  $\text{CH}_4$  production.

Values of  $\delta\text{mA}$  can be calculated by assuming a particular degradation pathway and fractionation factors known from microbial culture experiments and from studies of other methanogenic sediments. For example, we can assume that acetate is produced from organic carbon by fermenting bacteria, then the methyl group of acetate is further reduced to  $\text{CH}_4$  by acetoclastic methanogens. In marine sediments and rice field soil, the  $\delta^{13}\text{C}$  of the methyl group was found to be ~7‰ more negative than the  $\delta^{13}\text{C}$  of total acetate (Blair and Carter 1992; Sugimoto and Wada 1993). Because the  $\delta^{13}\text{C}$  of total acetate is usually close to that of organic matter, we can assume that the  $\delta^{13}\text{C}$  of the acetate methyl and carboxyl groups in Lake Kinneret have values of about –31 to –35‰ and –17 to –21‰, respectively. The  $\text{CH}_4$  produced from acetate should have an isotopic signature similar to the methyl group since acetate concentrations are low and turnover is fast in Lake Kinneret sediment. Under these conditions and when acetate is solely consumed by methanogens, very small or no difference in the  $\delta^{13}\text{C}$  has been observed between the methyl group of acetate and the acetate-derived  $\text{CH}_4$  (Sugimoto and Wada 1993). Therefore, the values of  $\delta\text{mA}$  expected for Lake Kinneret sediment should be only slightly more negative than the  $\delta^{13}\text{C}$  of –31 to –35‰ calculated for the acetate-methyl. These values are similar to  $\delta\text{mA}$  values

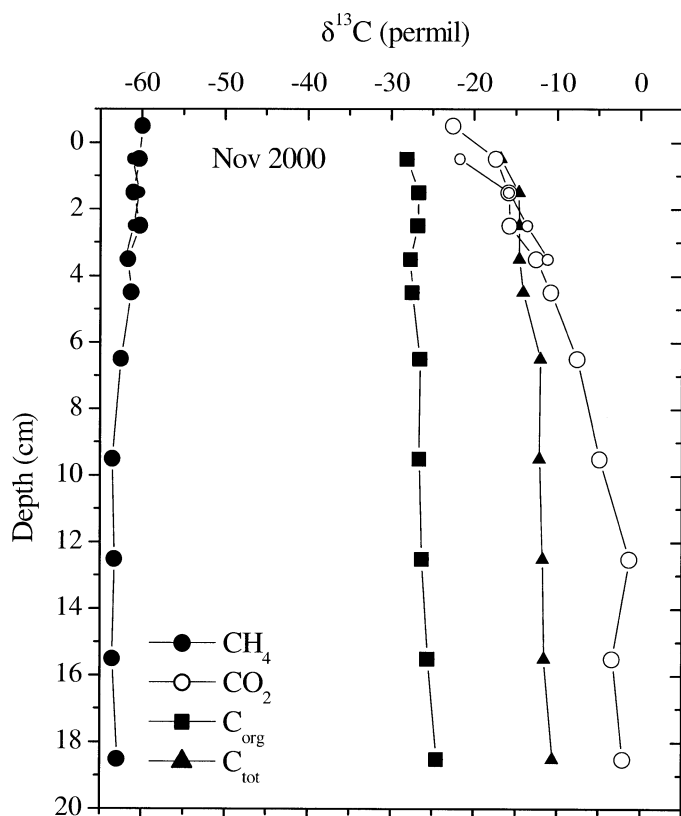


Fig. 4. Vertical profile of dissolved  $\delta^{13}\text{CH}_4$  and  $\delta^{13}\text{CO}_2$ , and  $\delta^{13}\text{C}_{\text{tot}}$  and  $\delta^{13}\text{C}_{\text{org}}$  in sediment sampled in November 2000. The smaller open and closed circles show data obtained from a parallel sediment core.

( $-30$  to  $-45\text{‰}$ ) in other methanogenic sediments (Alperin et al. 1992; Sugimoto and Wada 1993; Avery et al. 1999). These values are also in agreement with the upper range of  $\delta_{\text{ma}}$  values ( $< -34\text{‰}$ ) in Lake Kinneret, but are not in agreement with the lower range of  $\delta_{\text{ma}}$  values ( $> -62\text{‰}$ ).

Lower  $\delta_{\text{ma}}$  values require an additional fractionation step from the acetate-methyl to  $\text{CH}_4$ . Fractionation between acetate-methyl and  $\text{CH}_4$  was observed in marine sediments, in which the acetate was not only converted to  $\text{CH}_4$  but was also oxidized to  $\text{CO}_2$  by sulfate reducers (Blair and Carter 1992). In these sediments, the fractionation between acetate-methyl and  $\text{CH}_4$  was found to be about  $-32\text{‰}$ . If we adopt this fractionation value for Lake Kinneret sediment, the lower range of  $\delta_{\text{ma}}$  values ( $> -62\text{‰}$ ) would indeed be reached. However, since limiting acetate concentrations should prohibit any fractionation, the question remains—By which mechanism is this fractionation accomplished? It is also noteworthy that the fractionation by  $-32\text{‰}$  is even larger than the fractionation ( $-21\text{‰}$ ) observed in cultures of acetoclastic methanogens that are not acetate limited (Krzycki et al. 1987; Gelwicks et al. 1994). We hypothesize that the explanation for the unusually large fractionation during the conversion of acetate-methyl to  $\text{CH}_4$  is syntrophic acetate consumption (i.e., acetate is first oxidized to  $2\text{CO}_2 + 4\text{H}_2$ , and then one of the  $\text{CO}_2$ s is reduced by  $4\text{H}_2$  to  $\text{CH}_4$ ) (Zinder 1994). The exact  $^{13}\text{C}$  fractionation in the syntrophic pathway

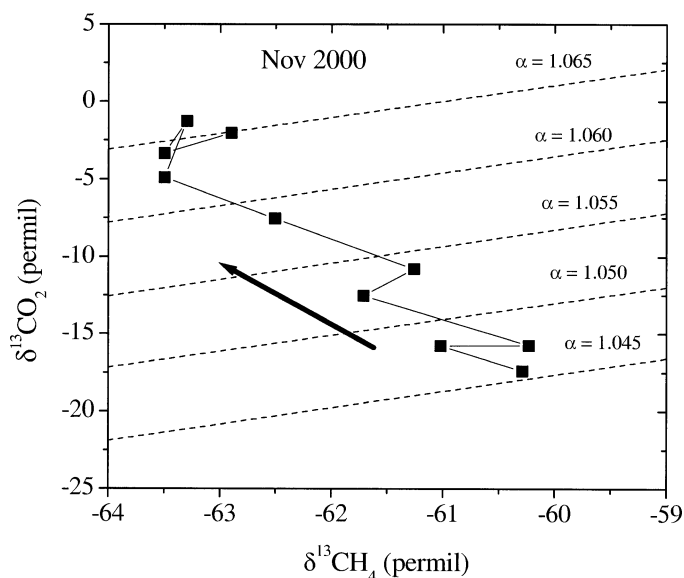


Fig. 5. Comparison of  $\delta^{13}\text{CO}_2$  and  $\delta^{13}\text{CH}_4$  dissolved in sediment sampled in November 2000, indicating a Type I distribution (Hornibrook et al. 2000) relative to the fractionation of  $\text{CO}_2$ -dependent methanogenesis  $\alpha_{\text{CO}_2}$ . The arrow indicates increasing sediment depth.

can hardly be predicted, since it is unknown how much the individual reaction steps would be limited by substrate availability and to what extent the common sedimentary  $\text{CO}_2$  pool, which is relatively enriched in  $^{13}\text{C}$  (about  $-5\text{‰}$ ), would exchange with the more depleted  $\text{CO}_2$  that is produced from syntrophic acetate oxidation. The  $\delta^{13}\text{C}$  of the syntrophically produced  $\text{CO}_2$  should be on the order of  $-17$  to  $-35\text{‰}$  when originating from the carboxyl and the methyl groups of acetate, respectively. It is likely that syntrophic acetate oxidation occurs within microbial consortia consisting of acetate oxidizers and  $\text{H}_2/\text{CO}_2$ -utilizing methanotrophs, thus allowing a spatially close transfer of  $\text{H}_2$  and  $\text{CO}_2$  between the syntrophic partners (Conrad et al. 1989). In this case, and with no  $\text{CO}_2$  exchange with the outside of the consortia, the  $\delta^{13}\text{C}$  of the  $\text{CH}_4$  molecules produced from the acetate-derived  $\text{CO}_2$  could be as low as  $-77$  to  $-105\text{‰}$ , assuming  $\alpha_{\text{CO}_2} = 1.06\text{--}1.07$ . With  $\text{CO}_2$  exchange, the overall fractionation would be smaller, but  $\delta_{\text{ma}}$  values of  $> -62\text{‰}$  still might be reached.

Measurements of  $^{13}\text{C}$  stable isotope signatures in vertical profiles of Lake Kinneret sediment and during incubation of sediment samples at  $15$  and  $30^\circ\text{C}$  showed distributions and trends that were not entirely consistent with the usual  $\text{CH}_4$  production pathways by  $\text{H}_2/\text{CO}_2$ -dependent and acetoclastic methanogenesis. We suggest that the isotopic patterns were affected by syntrophic acetate oxidation coupled to  $\text{H}_2/\text{CO}_2$ -dependent methanogenesis rather than by direct acetoclastic methanogenesis. This conclusion is consistent with previous results (Nüsslein et al. 2001). It is also consistent with incubation experiments of sediment samples from different depths, which consistently showed an increase of the apparent fractionation factor between  $\text{CO}_2$  and  $\text{CH}_4$ , a strong depletion of  $^{13}\text{C}$  in the  $\text{CH}_4$  produced, or both. An accurate quantification of the different methanogenic pathways is

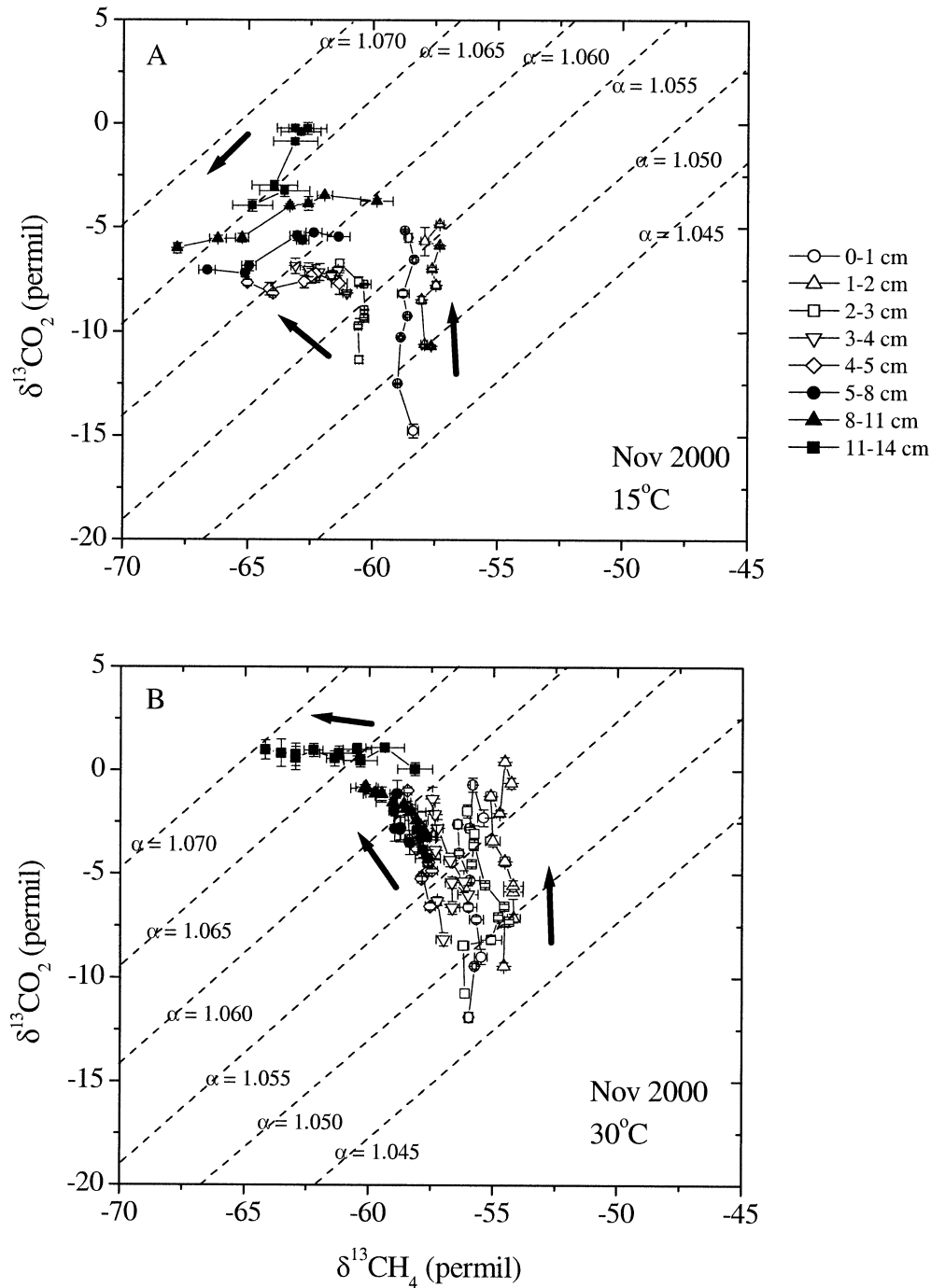


Fig. 6. Comparison of  $\delta^{13}\text{CO}_2$  and  $\delta^{13}\text{CH}_4$  produced after incubation of sediment sections at (A) in situ ( $15^\circ\text{C}$ ) temperature after and (B) elevated ( $30^\circ\text{C}$ ) temperature relative to the fractionation of  $\text{CO}_2$ -dependent methanogenesis ( $\alpha_{\text{CO}_2}$ ). The arrow indicates increasing time of incubation (<110 d). Data are means  $\pm$  SE of triplicate incubations.

presently hampered by the lack of data—in particular,  $\delta^{13}\text{C}$  values of the methyl and carboxyl groups of acetate. Furthermore, we do not know to what extent carbonate- and organic carbon-derived  $\text{CO}_2$  is exchanged. In particular, we do not know to what extent syntrophic acetate oxidation, which is processed by acetate-oxidizing bacteria and  $\text{CO}_2$ -reducing archaea, is functionally isolated within juxtaposed

microbial consortia (Conrad et al. 1989). The stable isotope biogeochemistry of  $\text{CH}_4$  in Lake Kinneret sediment is in many respects different from observations in other methanogenic wetlands (Hornibrook et al. 2000) and will be an interesting subject for future research. Another question is whether the observed stable carbon biogeochemistry is really unique to this lake or whether it also occurs in other

aquatic environments. Possibly, the  $\delta_{\text{ma}}$  values observed in marine sediments (Alperin et al. 1992; Blair and Carter 1992) are also influenced by syntrophic acetate oxidation. This speculation would be in agreement with the observation of pronounced homoacetogenic activity (Alperin et al. 1992) and the proposed role of reversed homoacetogenesis for syntrophic acetate oxidation (Zinder 1994).

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