APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Aug. 1984, p. 389–394
0099-2240/84/080389-06$02.00/0

Vol. 48, No. 2

Different Temperature Optima for Methane Formation When Enrichments from Acid Peat Are Supplemented with Acetate or Hydrogen

BO H. SVENSSON

Department of Microbiology, Swedish University of Agricultural Sciences, S-750 07 Uppsala, Sweden

Received 5 December 1983/Accepted 1 May 1984

Laboratory studies of methane formation in peat samples from an acid subarctic mire in Sweden indicated the presence of a low-temperature-adapted methanogenic flora. Enrichment culture studies with ethanol, acetate, hydrogen, or a combination of these as substrate for methane formation provided evidence for the existence of two different methanogenic populations in the peat: one, unaffected by hydrogen and using acetate, with a temperature optimum at 20°C; the other, oxidizing hydrogen, with a temperature optimum at ca. 28°C.

Release of methane into the atmosphere from the acid peat of the subarctic Stordalen mire in northern Sweden (68°22'N, 19°03'E) has been reported by Svensson (30–33; B. H. Svensson, Ph.D. dissertation, Swedish University of Agricultural Sciences, Uppsala, 1983) and B. H. Svensson and T. Rosswall (Oikos, in press). The mire was used as the Swedish International Biological Programme Tundra Biome Study site from 1970 to 1975 and is described by Rosswall et al. (27). The mire consists of a mosaic of elevated ombrotrophic areas, with hummocks and depressions, and minerotrophic depressions and pools. The pH of the peat varies from 3.5 to 5.0.

Despite the cold climate and acid conditions of the peat, the methane flux could be as much as 50% of the carbon (as CH_4 plus CO_2) leaving the mire and was strongly correlated to the moisture content of the peat (30; Svensson and Rosswall, in press). About 60% of the variation in methane emission rates over the season could be explained by temperature changes.

Methane production in relation to temperature in different ecosystems has been studied previously. Koyama (18) reported values of methane formation from rice paddy soils in Japan which had been incubated at 9.5 to 40°C in the laboratory. Four different soils were investigated. All of them showed optimal methane formation above 40°C, and the mean rates ranged from 0.1 to 2.8 ml of CH_4 per day and 100 g of soil (dry weight). He extrapolated these data to 0°C and also gave a value for 5°C (17). Atkinson and Hall (1) reported higher concentrations of methane at higher temperatures ranging from 7 to 28°C in water from a Georgia salt marsh. Zeikus and Winfrey (37) investigated sediments at three different depths from Lake Mendota, sampled in January and May, and incubated subsamples at 4 to 55°C. In all cases, optima were observed between 35 and 40°C. These optima were confirmed by ^{14}C studies. The maximum in situ temperature of the sediments was 23°C, and the methanogenic populations would thus never experience their optimum temperature range. These findings were supported by the investigation of Zinder and Brock (39), who observed the same optimum for conversion of methyl mercaptan to methane by sediments from Lake Mendota. According to Sandbeck and Ward (28), optimal methanogenesis in microbial mats of hot springs occurred between 50 and 60°C, although isolated *Methanobacterium thermoautotrophicum* strains showed optimum methanogenesis at 65°C.

A fourfold increase in the methane formation rate from acid peat incubated in the laboratory at 27°C as compared with 10°C was reported by King et al. (16). Kelly and Chynoweth (14) performed studies on the dependence of methane production on temperature in sediments. They obtained Q_{10} values of 0.83 to 5.7, depending on the temperature interval considered. Higher Q_{10} values were generally obtained for low-temperature intervals than for high-temperature intervals.

Most methanogens so far studied in pure culture are mesophilic (20), but several thermophilic strains have been isolated (8). Two of the latter have been shown to have lower optima (between 20 and 25°C) as compared with the others (between 30 and 40°C). These species belong to the genus *Methanogenium* and were isolated from sediment cores from the Black Sea and the Cariaco Trench (25). These habitats are the only ones so far shown to have a low-temperature-adapted methanogenic flora, although several of the habitats where methanogens have been studied always had temperatures below 10°C. In fact, thermophilic strains have been isolated from such habitats as well (26). Williams (34) briefly mentioned the probability that peat methanogenic bacteria may be adapted to low temperatures but gave no supporting evidence.

The methanogenic bacteria are able to utilize hydrogen, acetate, formate, methanol, and methylamines as electron donors (2). Other products from anaerobic decomposition of organic matter have to be converted to these compounds to be reduced to methane. Associations of bacteria performing such conversions and methanogens have been shown to have the capacity to decompose several organic substances to acetate and methane (13, 24). The pioneering work in this area was done by Bryant et al. (6) and showed that the decomposition of ethanol by *Methanobacillus omelianskii* was in fact performed by two bacteria. One oxidized ethanol to acetate and hydrogen, and the other oxidized hydrogen to methane.

It has been shown that anaerobic cellulose decomposers isolated from peat can produce ethanol and acetate (12) and that enrichments of peat with ethanol show methane formation (Svensson, Ph.D. dissertation). Ethanol, acetate, and hydrogen were used in further studies described in this paper on the effects of substrate additions and temperature on methane production from acid peat incubated in the laboratory.

MATERIALS AND METHODS

The peat used in the experiments was collected in the autumn from a minerotrophic depression, with vegetation

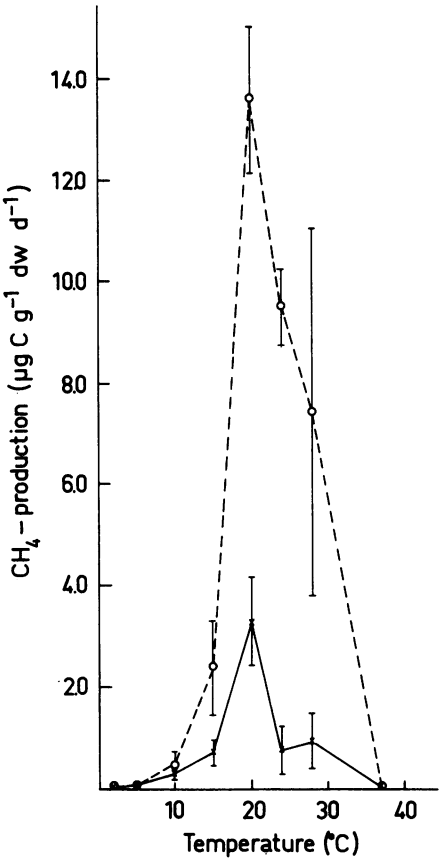

FIG. 1. Methane formation from acid peat incubated with a headspace of H_2-CO_2 (4:1) at different temperatures for 1 (——) and 2.5 months (– –). Bars, Standard deviations for the means of three replicates. dw, Dry weight. The peat had been stored at 5°C with the same headspace.

consisting of *Sphagnum lindbergii* Schimp. and *Eriophorum angustifolium* Honck. The pH varied from 4.2 to 4.9, and the water table was close to the surface. The permafrost level was ca. 90 cm below the surface at the time of sampling. Peat cores were taken by means of a Holmen auger (7). The cores were subdivided, and the samples were transferred to bottles while being flushed with nitrogen to avoid oxygen contamination. The bottles were transported to the laboratory at <5°C.

Experiment 1. Peat samples from the most active layers (10 to 15 cm), incubated with a headspace of H_2-CO_2 (4:1) at 5°C for about 3 months, were pooled and thoroughly mixed. The mixture was redistributed in 125-ml flasks, which were flushed with H_2-CO_2 (4:1). Three replicates were placed at 2, 5, 10, 15, 20, 24, 28, and 37°C.

Experiment 2. Peat which had been collected from the same layers as above and stored with N_2 at 2°C for 6 months was also incubated for temperature studies as above, but, in addition, a treatment with a headspace of N_2-CO_2 (4:1) and an incubation temperature of 12°C was included.

The samples from experiments 1 and 2 were incubated and analyzed for methane formed after 1 and 2.5 months. Before analyses, the flasks were flushed with the corresponding gas mixture and shaken vigorously to obtain an equilibrium, after which the methane concentration was determined. After a week of further incubation, the methane accumulation was measured. Thus, the reported daily rates are means over the 7-day interval before sampling. One milliliter of gas was withdrawn and analyzed on a Varian Aerograph 600D equipped with a flame ionization detector and a column with Porapak T.

Experiment 3. Peat from three cores (diameters of 113 mm) was subsampled at 5, 10, 15, 20, 25, and 30 cm below the surface. The samples from each level were mixed in a bottle in the field, which was continuously flushed with Ar (O_2 < 0.1 ppm). The bottles were brought to the laboratory at a temperature of <5°C. All bottles and solutions were kept cool during the work to avoid any temperature adaptation of the microbial populations. All transfers within the laboratory were carried out by the Hungate technique (9), as modified by Balch and Wolfe (3). Resazurin (1 ppm [wt/vol]) was included as an indicator for reduced substrates and solutions. Peat (50 g) from each level was homogenized in 100 ml of a prereduced sterile solution of 0.1% Trypticase (BBL Microbiology Systems, Cockeysville, Md.) and 0.1% yeast extract (Oxoid Ltd., London, England) in an Automix blender (Hugo Tillquist AB, Stockholm, Sweden). The blender and the solution were precooled in ice water, and the temperature never exceeded 7°C after the homogenization. The pH of the homogenates was 4.0 to 4.1.

Inoculates of 1 ml were transferred to serum bottles (n = 96) with 10 ml of prereduced substrate. The maintenance medium used by Zeikus and Wolfe (38) was supplemented with either 210 µmol of ethanol or 210 µmol of acetate–0.1% Trypticase–0.1% yeast extract (final concentration). Na_2CO_3 was omitted since the pH had been adjusted to 5.0 before being autoclaved and was 4.8 after being sterilized; under these conditions most of the carbonate would have been converted to CO_2. The reducing agent was added after sterilization. The medium was also supplemented with 0.1 ml of a FeS suspension prepared according to Brock and O'Dea (4).

The inoculated bottles were stored for 2 days at 5°C before the headspace of N_2-CO_2 was changed to H_2-CO_2 in half of the ethanol and acetate bottles. Two bottles from each treatment were then incubated for a month at the same temperatures as the peat samples in experiment 1 and analyzed as above.

RESULTS

Methane formation versus temperature for the incubations of peat in experiments 1 and 2 are shown in Fig. 1 and 2. The peat previously exposed to H_2-CO_2 at 5°C (experiment 1; Fig. 1) and that stored with N_2 (experiment 2; Fig. 2) both showed activity peaks at 20°C after 1 month of incubation. Another peak was indicated at about 28°C in experiment 1 (Fig. 1) and with N_2 in experiment 2 (Fig. 2), although great variations were obtained. After 1 month, the methane formation was about the same at 15°C or below for incubations with N_2 or H_2, but lower with H_2 than N_2 at 20°C and above (experiment 2). The rates in experiment 1 increased with further incubation for 1.5 months, especially at 24 and 28°C (more than 10 times, compared with 5 times at 20°C and 3 times at 15°C) (Fig. 1). The same rates prevailed up to 12 to 15°C with H_2 and to 12°C with N_2 1.5 months later (experiment 2; Fig. 2). At 15°C, with N_2, the formation rate was 1.7 times greater after 2.5 months as compared with after 1 month, whereas about the same rate was observed at 20°C. At 24°C, the rates were much greater after 2.5 months, and the activity peak occurred at this temperature. The rates with H_2 were higher than those with N_2 at the higher temperatures.

Methane accumulation from peat inocula incubated with

acid substrate (pH 4.8; experiment 3) is shown in Fig. 3. The values of the two replicates ranged up to one-third around the means shown. With substrate including acetate and with N_2 as the headspace, an activity peak seemed to occur at about 20°C for the peat from depths of 10, 15, and 20 cm. For the 5-cm samples, the peak seemed to be at ca. 15°C. With H_2, the activity peaks were shifted to 24 to 28°C, with rates being an order of magnitude higher than at the corresponding temperatures with N_2. Except for the 5-cm level, the rates at 20°C and below were only slightly affected by the change in headspace gas. For the lower strata, the rates were higher with H_2-CO_2.

When the substrate was supplemented with ethanol (Fig. 3c and d), two peaks were observed for the 5-cm level at 20 and 28°C, respectively. With peat from lower strata, lower rates and a shift of the activity peak towards higher temperatures seemed to occur. With H_2 (Fig. 3d), lower rates were obtained than with N_2 below 20°C, and the highest accumulation seemed to be between 24 and 28°C, similar to that for acetate with H_2 (Fig. 3b).

DISCUSSION

The results of the two experiments with peak incubated at different temperatures (Fig. 1 and 2) showed that a comparatively low temperature optimum may exist for the peat methanogenic flora. The low optimum was similar to that obtained for *Methanogenium* sp. (25) but lower than those previously reported for other methanogenic bacteria. It is, however, not necessarily the methanogenic bacteria in these samples that showed a low-temperature optimum; any of the microorganisms involved in the decomposition of peat to acetate, carbon dioxide, and hydrogen may cause the patterns observed. On prolonged incubation of the peat above 10 to 12°C, bacteria with higher temperature optima were obviously enriched. These seemed to be stimulated by the presence of hydrogen, since the rate increase at 28°C with H_2 was much greater than that with N_2. Thus, from these experiments, it seemed probable that two bacterial populations with different temperature optima were involved in methane formation from peat. The different rates may depend on differences between the inocula, one being previously stored under hydrogen and the other under nitrogen (Fig. 1 and 2).

The enrichment studies with acetate and ethanol furnished further support for the existence of two methanogenic populations. Peat and acetate medium with N_2-CO_2 or H_2-CO_2 also indicated two different optima (at ca. 20 and 28°C), which may be the result of two different methanogenic populations: one used acetate and the other hydrogen and carbon dioxide as substrates for methane formation. Although the variation was high, the explanation seems justified by the repeated pattern for at least the three upper layers

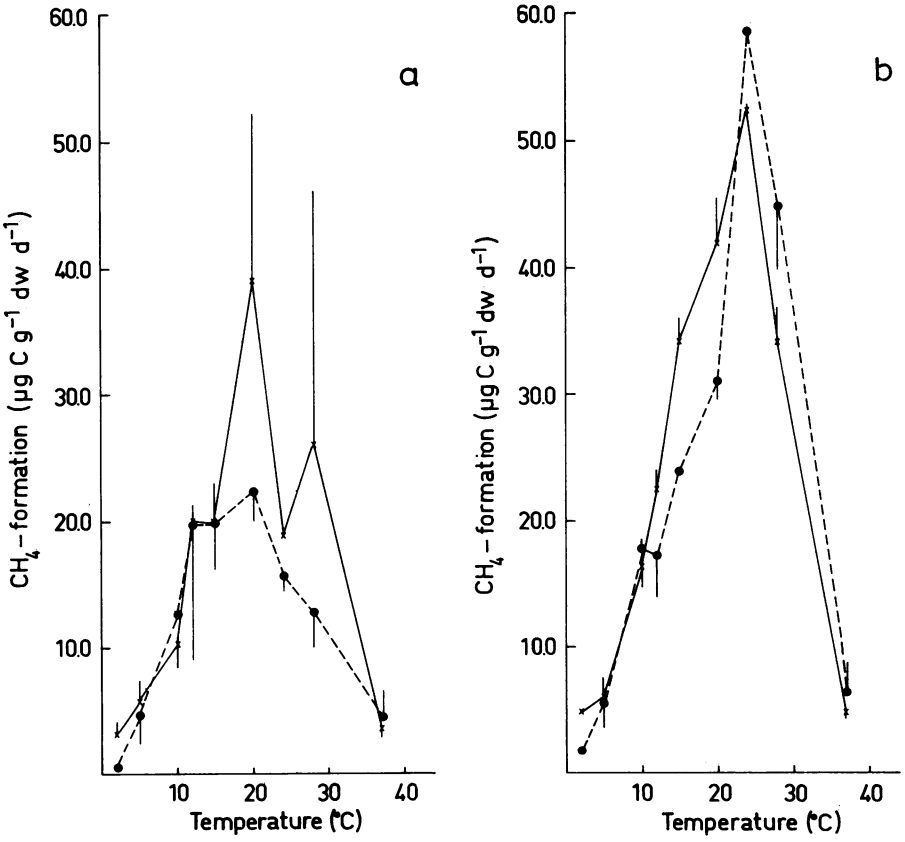

FIG. 2. Methane formation from acid peat, with a headspace of H_2-CO_2 (——) or N_2-CO_2 (— —) (4:1) incubated at different temperatures for 1 (a) and 2.5 (b) months. Bars, Standard deviations for the means of three replicates. dw, Dry weight. The peat had been stored at 2°C with oxygen-free N_2 as the headspace.

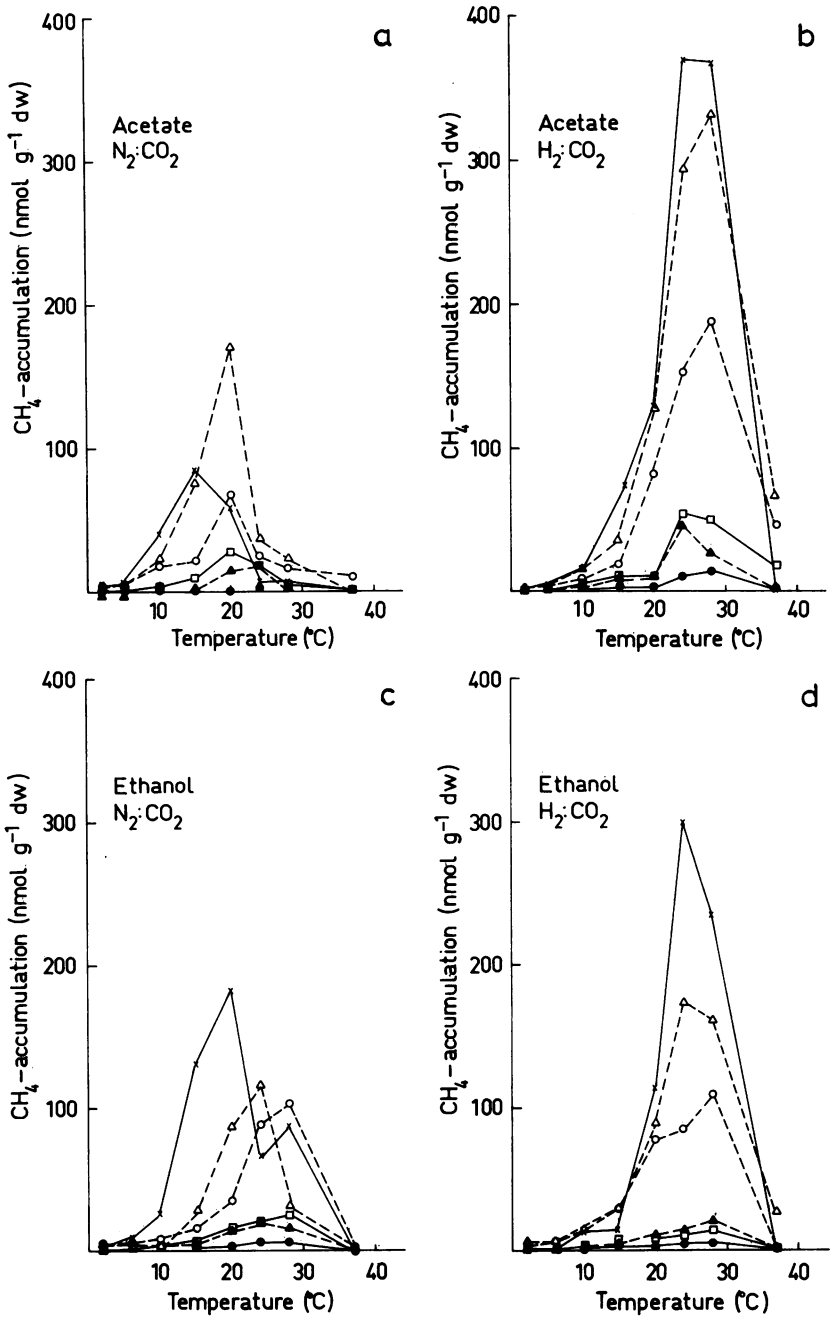

FIG. 3. Methane accumulation from enrichment cultures of acid peat from different depths (in centimeters) below the surface: (x) 5, (△) 10, (○) 15, (□) 20, (▲) 25, and (●) 30, incubated at different temperatures. The media contained acetate (a and b) or ethanol (c and d) and headspaces of N_2 (a and c) or H_2 (b and d) mixed with CO_2 (4:1). dw, Dry weight. The values are means of two replicates, and the range around the means could be plus or minus one-third of the mean. The cultures were run for 1 month.

of peat. The accumulation at and below 20°C seemed to be the same with H_2 or N_2, an observation also made by Khan and Mes-Hartree (15) for enrichment cultures on cellulose. They had microscopic evidence that their cultures consisted of a methanogen similar to that described by Zehnder et al. (35). The latter had isolated *Methanobacterium soehngenii*, which, when using acetate for methane formation, was unaffected by hydrogen. This isolate was later renamed *Methanothrix soehngenii* (10). Since the acetate-utilizing methanogens in the peat seemed to be unaffected by hydrogen, *Methanothrix soehngenii* may be involved in the methane formation at low temperature.

Other methanogens so far known to use acetate are *Methanosarcina barkeri* (2) and *Methanococcus mazei* (21). Hydrogen inhibits methane formation from acetate by *Methanosarcina barkeri* (11, 21, 29) and by some strains of *Methanococcus mazei* (19). Thus, it seems unlikely that *Methanosarcina* sp. take part in methane production at the low temperatures of the peat enrichment cultures, but *Methanococcus* sp. may be involved.

Ethanol per se cannot be used by any methanogen but may be decomposed by other organisms to acetate and hydrogen in a synergistic association with methanogens (6). Methanogens oxidize the hydrogen, which would otherwise rapidly inhibit the ethanol degradation to acetate and hydrogen. The same type of synergism, as for the S organism and *Methanobacterium* sp. strain MOH for decomposing ethanol, was shown to exist for *Desulfovibrio* sp. when grown together with a hydrogen-oxidizing methanogen similar to *Methanobacterium formicium* (5). This association only worked at low sulfate concentrations, since sulfate promotes the growth of *Desulfovibrio* sp. and is an energetically favored sink for hydrogen. Since it had earlier been shown that ethanol stimulated methane formation from Stordalen peat (Svensson, Ph.D. dissertation), this compound was used in a parallel study. Sulfate concentrations were comparatively low at the site (22). Sulfate reducers have been enriched from the peat (Svensson, Ph.D. dissertation) and may thus be associated with methanogens. Ethanol was also shown to be one of the degradation products obtained with cellulose-decomposing anaerobes from Finnish peats (12).

The results from the ethanol enrichments were not as clear cut, although those with H_2 clearly showed optima at 24 to 28°C similar to results from the acetate experiment. The optima for methane production from samples from the three upper peat levels incubated with ethanol (with N_2) were higher at lower depths. This might be due to the activity of the ethanol-decomposing organisms at the different depths and also to interference by the acetate-using methanogens with a lower optimum than for those using hydrogen. Acetate was presumably produced from the decomposition of ethanol. The presence of hydrogen in the cultures with ethanol caused decreased methane accumulation at 15 and 20°C for the 5-cm level, whereas a slight increase was obtained for the 10- and 15-cm levels. This suggested that the ethanol decomposition was inhibited by hydrogen in peat from the upper layer. The low-temperature optimum with nitrogen was probably due to methane formation by the acetate-utilizing population (Fig. 3c).

Methane production with hydrogen showed higher rates with acetate present for all levels compared with that with ethanol. This suggested a stimulatory effect on hydrogen oxidation by acetate since ethanol decomposition to acetate and hydrogen will be inhibited by the headspace hydrogen. This is in accordance with the findings of Zeikus et al. (36).

In summary, incubation of bulk peat as well as subsamples in enrichment cultures at different temperatures showed the presence of low-temperature-adapted methanogens with an optimum at about 20°C and another population with a higher optimum (between 24 and 28°C). The two seemed to occupy two nutritional niches: the low-temperature population used acetate for methane formation, and the one with the higher optimum oxidized hydrogen. The shift in optimum toward higher temperatures with prolonged incubation of the bulk peat samples could be explained by the different substrates used for methane production. The population using hydrogen is more likely to be enriched compared with the one using acetate, since the former substrate is energetically more favorable (23). The hydrogen oxidizers would, therefore, interfere with the acetate-using methanogens, giving a mixed optimum.

ACKNOWLEDGMENTS

I thank T. Rosswall, Department of Microbiology, Swedish University of Agricultural Sciences, for valuable discussion all through the work and preparation of the manuscript. Thanks are also given to H. Ljunggren, Department of Microbiology, Swedish University of Agricultural Sciences, P. Rönnow, Department of Marine Microbiology, University of Gothenburg, and D. D. Wynn-Williams, British Antarctic Survey, Cambridge, United Kingdom, for constructive criticism of the manuscript and also to R. Kumar, who corrected the manuscript for grammar.

This work was a part of the projects in contracts no. B 3358-002 and B 0313-037 with the Swedish Natural Science Research Council.

LITERATURE CITED

1. **Atkinson, L. P., and J. R. Hall.** 1976. Methane distribution and production in the Georgia salt marsh. Estuarine Coastal Mar. Sci. **4**:677–686.
2. **Balch, W. E., G. E. Fox, L. J. Magrum, C. R. Woese, and R. S. Wolfe.** 1979. Methanogens: reevaluation of a unique biological group. Microbiol. Rev. **43**:260–296.
3. **Balch, W. E., and R. S. Wolfe.** 1976. New approach to the cultivation of methanogenic bacteria: 2-mercaptoethanesulfonic acid (HS-CoM)-dependent growth of *Methanobacterium ruminantium* in a pressurized atmosphere. Appl. Environ. Microbiol. **32**:781–791.
4. **Brock, T. D., and K. O'Dea.** 1977. Amorphous ferrous sulfide as a reducing agent for culture of anaerobes. Appl. Environ. Microbiol. **33**:254–256.
5. **Bryant, M. P., L. L. Campbell, C. A. Reddy, and M. R. Crabill.** 1977. Growth of desulfovibrio in lactate or ethanol media low in sulfate in association with H_2-utilizing methanogenic bacteria. Appl. Environ. Microbiol. **33**:1162–1169.
6. **Bryant, M. P., E. A. Wolin, M. J. Wolin, and R. S. Wolfe.** 1967. *Methanobacillus omelianskii*, a symbiotic association of two species of bacteria. Arch. Microbiol. **59**:20–31.
7. **Holmen, H.** 1964. Forest ecological studies on drained peat land in the province of Uppland, Sweden. I-III. Stud. For. Suec. **16**:1–236.
8. **Huber, H., M. Thomm, H. König, G. Thies, and K. O. Stetter.** 1982. *Methanococcus thermolitotrophicus*, a novel thermophilic lithotrophic methanogen. Arch. Microbiol. **132**:47–50.
9. **Hungate, R. E.** 1950. The anaerobic mesophilic cellulolytic bacteria. Bacteriol. Rev. **14**:1–49.
10. **Huser, B. A., K. Wuhrman, and A. J. B. Zehnder.** 1982. *Methanotrix soehngenii* gen. nov., sp. nov., a new acetotrophic non-hydrogen-oxidizing methane bacterium. Arch. Microbiol. **132**:1–9.
11. **Hutten, T. J., H. C. M. Bongaerts, C. van der Drift, and G. D. Vogels.** 1980. Acetate, methanol and carbon dioxide as substrates for growth of *Methanosarcina barkeri*. Antonie van Leeuwenhoek J. Microbiol. **46**:601–610.
12. **Isotalo, A.** 1951. Studies on the ecology and physiology of cellulose-decomposing bacteria in raised bogs. Acta Agral. Fenn. **74**:1–108.
13. **Kaiser, J. P., and K. W. Hanselmann.** 1982. Fermentative metabolism of substituted monoaromatic compounds by a bacterial community from anaerobic sediments. Arch. Microbiol. **133**:185–194.
14. **Kelly, C. A., and D. P. Chynoweth.** 1981. The contributions of temperature and of the input of organic matter in controlling rates of sediment methanogenesis. Limnol. Oceanogr. **26**:891–897.
15. **Khan, A. W., and M. Mes-Hartree.** 1981. Metabolism of acetate and hydrogen by a mixed population of anaerobes capable of converting cellulose to methane. J. Appl. Bacteriol. **50**:283–288.
16. **King, G. M., T. Berman, and W. J. Wiebe.** 1981. Methane formation in the acidic peats of Okefenokee Swamp, Georgia. Am. Midl. Nat. **105**:386–389.
17. **Koyama, T.** 1963. Gaseous metabolism in lake sediments and paddy soils and the production of atmospheric methane and hydrogen. J. Geophys. Res. **68**:3971–3973.
18. **Koyama, T.** 1964. Gaseous metabolism in lake sediments and paddy soils, p. 363–375. *In* V. Colombo and G. Hobson (ed.), Advances in organic geochemistry. MacMillan, Inc., New York.
19. **Mah, R. A.** 1980. Isolation and characterization of *Methanococcus mazei*. Curr. Microbiol. **3**:321–326.
20. **Mah, R. A., and M. R. Smith.** 1981. The methanogenic bacteria, p. 948–977. *In* M. P. Starr, H. Stolp, H. G. Trüper, A. Balows,

and H. G. Schlegel (ed.), The prokaryotes. Springer-Verlag KG, Berlin.
21. **Mah, R. A., M. R. Smith, and L. Baresi.** 1978. Studies on an acetate-fermenting strain of *Methanosarcina*. Appl. Environ. Microbiol. **35**:1174–1184.
22. **Malmer, N., and B. Nihlgårdh.** 1980. Supply and transport of mineral nutrients in a subarctic mire. Ecol. Bull. N.F.R. (Naturvetensk. Forskningsrådet) **30**:63–95.
23. **McInerney, M. J., and M. P. Bryant.** 1981. Anaerobic degradation of lactate by syntrophic associations of *Methanosarcina barkeri* and *Desulfovibrio* species and effect of H_2 on acetate degradation. Appl. Environ. Microbiol. **41**:346–354.
24. **McInerney, M. J., M. P. Bryant, and N. Pfennig.** 1979. Anaerobic bacterium that degrades fatty acids in syntrophic association with methanogens. Arch. Microbiol. **122**:129–135.
25. **Romesser, J. A., R. S. Wolfe, F. Mayer, E. Spiess, and A. Walther-Mauruschat.** 1979. *Methanogenium*, a new genus of marine methanogenic bacteria, and characterization of *Methanogenium cariaci* sp. nov. and *Methanogenium marisnigri* sp. nov. Arch. Microbiol. **121**:147–153.
26. **Rönnow, P. H., and L. Å. H. Gunnarsson.** 1981. Sulfide-dependent methane production and growth of a thermophilic methanogenic bacterium. Appl. Environ. Microbiol. **42**:580–584.
27. **Rosswall, T., J. G. K. Flower-Ellis, L. G. Johansson, S. Jonsson, B. E. Rydén, and M. Sonesson.** 1975. Stordalen (Abisko), Sweden. Ecol. Bull. N.F.R. (Naturvetensk Forskningsrådet) **20**:265–294.
28. **Sandbeck, K. A., and D. M. Ward.** 1982. Temperature adaptations in the terminal processes of anaerobic decomposition of Yellowstone National Park and Icelandic hot spring microbial mats. Appl. Environ. Microbiol. **44**:844–851.
29. **Smith, M. R., and R. A. Mah.** 1978. Growth and methanogenesis by *Methanosarcina* strain 227 on acetate and methanol. Appl. Environ. Microbiol. **36**:870–879.
30. **Svensson, B. H.** 1976. Methane production in tundra peat, p. 135–139. *In* H. G. Schlegel, G. Gottschalk, and N. Pfennig (ed.), Microbial production and utilization of gases (H_2, CH_4, CO). E. Goltze KG, Göttingen, Federal Republic of Germany.
31. **Svensson, B. H.** 1980. Carbon dioxide and methane fluxes from the ombrotrophic parts of a subarctic mire. Ecol. Bull. N.F.R. (Naturvetensk. Forskningsrådet) **30**:235–250.
32. **Svensson, B. H., and T. Rosswall.** 1980. Energy flow through the subarctic mire at Stordalen. Ecol. Bull. N.F.R. (Naturvetensk. Forskningsrådet) **30**:283–301.
33. **Svensson, B. H., A. K. Veum, and S. Kjelvik.** 1975. Carbon losses from tundra soils, p. 279–286. *In* F. E. Wielgolaski (ed.), Fennoscandian tundra ecosystems, part 1: plants and microorganisms. Springer-Verlag KG, Berlin.
34. **Williams, R. T.** 1980. Utilization of the peatland methanogenic microflora, p. 495–505. *In* D. V. Punwani and J. W. Weatherly III (ed.), Peat as an alternative energy. IGT, Chicago.
35. **Zehnder, A. J. B., B. A. Huser, T. D. Brock, and K. Wuhrmann.** 1980. Characterization of an acetate-decarboxylating, non-hydrogen-oxidizing methane bacterium. Arch. Microbiol. **124**:1–11.
36. **Zeikus, J. G., P. J. Wiemer, D. R. Nelson, and L. Daniels.** 1975. Bacterial methanogenesis: acetate as a methane precursor in pure culture. Arch. Microbiol. **104**:129–134.
37. **Zeikus, J. G., and M. R. Winfrey.** 1976. Temperature limitation of methanogenesis in aquatic sediments. Appl. Environ. Microbiol. **31**:99–107.
38. **Zeikus, J. G., and R. S. Wolfe.** 1972. *Methanobacterium thermoautotrophicus* sp. n., an anaerobic, autotrophic, extreme thermophile. J. Bacteriol. **109**:707–713.
39. **Zinder, S. H., and T. D. Brock.** 1978. Production of methane and carbon dioxide from methane thiol and dimethyl sulphide by anaerobic lake sediments. Nature (London) **273**:226–228.