Carbon and hydrogen isotope systematics of bacterial formation and oxidation of methane

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Abstract

The diagenetic cycling of carbon within recent unconsolidated sediments and soils generally can be followed more effectively by discerning changes in the dissolved constituents of the interstitial fluids, rather than by monitoring changes in the bulk or solid organic components. The major dissolved carbon species in diagenetic settings are represented by the two carbon redox end-members CH4 and CO2. Bacterial uptake by methanogens of either CO2 or ‘‘preformed’’ reduced carbon substrates such as acetate, methanol or methylated amines can be tracked with the aid of carbon (13C/12C) and hydrogen (D/H = 2H/1H) isotopes. The bacterial reduction of CO2 to CH4 is associated with a kinetic isotope effect (KIE) for carbon which discriminates against 13C. This leads to carbon isotope separation δ13C exceeding 9524 C and gives rise to δ13C(CH4) values as negative as −110‰ vs. PDB. The carbon KIE associated with fermentation of methylated substrates is lower (εC is ca. 40 to 60, with δ13C(CH4) values of −50‰ to −60‰). Hydrogen isotope effects during methanogenesis of methylated substrates can lead to deuterium depletions as large as δD(CH4) = −531‰ vs. SMOW, whereas, bacterial D/H discrimination for the CO2-reduction pathway is significantly less (δD(CH4) ca. −170‰ to −250‰). These field observations have been confirmed by culture experiments with labeled isotopes, although hydrogen isotope exchange and other factors may influence the hydrogen distributions. Bacterial consumption of CH4, both aerobic and anaerobic, is also associated with KIEs for C and H isotopes that enrich the residual CH4 in the heavier isotopes. Carbon fractionation factors related to CH4 oxidation are generally less than εC = 10, although values > 20 are known. The KIE for hydrogen (εD) during aerobic and anaerobic CH4 oxidation is between 95 and 285. The differences in C and H isotope ratios of CH4, in combination with the isotope ratios of the coexisting H2O and CO2 pairs, differentiate the various bacterial CH4 generation and consumption pathways, and elucidate the cycling of labile sedimentary carbon. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

The interplay between bacterial processes of methanogenesis and methanotrophy continues to attract the interest of microbiologists and geochemists, despite the fact that CH4, even at saturated concentrations in shallow organic-rich sediments, represents only about 1‰ of the total organic carbon present (e.g., 10 m water depth, ca. 4% Corg, porosity ca. 0.9). Apart from the more fundamental interests,
such as bacterial physiology or organic matter remineralization, some of this curiosity reflects the economic fact that roughly 20% of the worldwide natural gas reservoirs are from bacterial sources (Rice, 1992; Rice and Claypool, 1981; Whiticar, 1994). The bacterial CH₄ cycle also receives current attention owing to its impact on the atmospheric CH₄ budget (Khalil and Shearer, 1993). This follows the identification that CH₄ in the atmosphere is: (1) a significant radiatively active or “greenhouse” gas, (2) represents an important sink for tropospheric hydroxyl radicals, (3) increasing in tropospheric concentration at a rate of ca. 18 to 9 ppb yr⁻¹ (e.g., Dlugokencky et al., 1995), and that (4) bacterial CH₄ is the primary source (ca. 80%-90%) of this atmospheric CH₄ (e.g., Cicerone and Oremland, 1988; Whiticar, 1993).

Furthermore, the processes of methanogenesis and aerobic CH₄ oxidation are also attractive to study by microbiologists because they involve only single or two-carbon reactions. Thus, these bacterial pathways are more easily followed and reproduced in the laboratory. For the geochemist, methanogenesis and methanotrophy are interesting because they are sensitive indicators of the state of diagenesis in a particular environmental setting.

The interest expressed in this paper reflects the intricate diagenetic relationships in the carbon cycle that permit the most oxidized form of carbon to reside and be maintained adjacent to its most reduced form (Fig. 1). The physical (depth) separation of CH₄ formation and consumption is often a transitional feature rather than distinctly zoned. The activity maxima of the respective CH₄ formation and consumption processes can be separated spatially from each other by several decimeters in depth, as in marine sediments, or at a sub-millimeter scale, as in certain stromatolitic hydrothermal or hot spring environments (e.g., Kuhl and Barker Jørgensen, 1992).

An elegant method to track the complimentary processes of methanogenesis and methanotrophy is provided by the stable isotopes of C and H. The partitioning of the light and heavy isotopes of hydrogen and carbon (e.g., Fig. 1) and the resultant isotope signatures can be diagnostic for the identification of methane type and pathway. This paper reviews the stable isotope systematics primarily associated with the incorporation and release of C and H during methanogenesis and methylotrophy, i.e., the bacterial formation and consumption of CH₄.

2. Methanogenic pathways

The microbiology, ecology and biochemistry of the various bacterial CH₄ formation pathways have been reviewed extensively in several monographs (e.g., Oremland and Capone, 1988; Zehnder, 1988; Garcia, 1990; König, 1992; Marty, 1992). Methanogens, fermentative archaeabacteria, are obligate anaerobes that metabolize only in anoxic conditions at redox levels Eh < −200 mV. The fact that
they cannot tolerate significant $pO_2$, nitrate or nitrite levels are due primarily to the instability of their $F_{420}$-hydrogenase enzyme complex (Schönheit et al., 1981). Methanogens form methane by pathways that are commonly classified to the type of carbon precursor utilized by them. The primary methanogenic pathways are referred to as: (1) hydrogenotrophic – 52 species described, (2) acetotrophic – 19 species described and (3) methylotrophic – 10 species described (e.g., Conrad et al., 1985). Hydrogeno-methylotrophic and alcoholotrophic methanogens have also been discussed (Neue and Roger, 1993). It is important to remember that the anaerobic remineralization of organic matter, ultimately to methane, relies on consortia of bacteria and microbes that successively break down larger molecules. Conrad (1989) recognized that four bacterial assemblages operate in this complementary fashion: (1) hydrolytic and fermenting bacteria, (2) $H^+$-reducing bacteria, (3) homoacetogenic bacteria and finally (4) methanogenic bacteria.

An approach, alternative to biochemistry, to classify methanogenic pathways uses microbial ecological relationships, namely methanogenic substrates. Methanogens utilize relatively few and simple compounds to obtain energy and cell carbon: (1) competitive and (2) non-competitive. The former are those substrates which can be utilized more effectively by other bacterial assemblages, such as sulphate reducing bacteria (SRB), and thus are either made unavailable (out-competed) or severely restricted to the methanogens. Non-competitive substrates are those which are more suitable to methanogens and less attractive to other bacteria, e.g., SRBs. Other factors, including redox potential, the availability of nutrients, substrates and terminal electron acceptors, or consumption reactions can determine the occurrence of bacterial methane in a particular environment. These competitive and non-competitive methanogenic pathways are outlined below.

2.1. Competitive substrates

Competitive substrates include $CO_2$ (reduced by hydrogen), acetate and formate, i.e., hydrogenotrophic, acetotrophic and methylotrophic pathways. The first one, termed the “carbonate reduction” pathway, can be represented by the general reaction:

$$CO_2 + 8H^+ + 8e^- \rightarrow CH_4 + 2H_2O,$$

and the net reaction for the acetate fermentation pathway is:

$$^*CH_3COOH \rightarrow ^*CH_4 + CO_2.$$
Fig. 2. Schematic diagram showing methanogenic pathways in marine and terrestrial environments.

important substrates for methanogenesis (Fig. 2, e.g., Marty, 1992). Methanogenesis by carbonate reduction in freshwater environments is initially less significant, but increases in importance as the other substrate pools become exhausted, and the methanogens (excepting the obligate methylotrophs) switch over to or start reducing the bicarbonate carbon source with hydrogen. Previous investigators stated that acetate fermentation is responsible for roughly 70% of the methanogenesis in freshwater environments, e.g., Koyama (1964), Takai (1970), Belyaev et al. (1975), Winfrey et al. (1977) and Cappenberg and Jongejan (1978). The utilization of other methylated substrates and/or carbonate reduction are suggested as constituting the remainder. Similar results have been cited for sewage sludges and from culture studies (Bryant, 1979; Zehnder et al., 1982).

2.2. Non-competitive substrates

Non-competitive substrates for methanogenesis include both the acetotrophic and methylotrophic pathways that utilize the carbon substrates methanol, methylated amines, such as mono-, di- and tri-methylamines (MA, DMA and TMA, respectively), and certain organic sulphur compounds, e.g., dimethylsulphide (DMS; Daniels et al., 1984; Kiene et al., 1986). A typical fermentation reaction for these substrates by methylotrophic methanogens can generally be represented by:

\[
\text{CH}_3 - A + \text{H}_2\text{O} \rightarrow \text{CH}_4 + \text{CO}_2 + A - \text{H},
\]
Fig. 3. Sediment depth profiles of methane concentration and carbon isotope composition in marine and freshwater environments. A = oxic zone, B = sulphate reduction zone, C = main methanogenic zone, D = zone of substrate depletion and/or shift to alternative substrate for methanogenesis, e.g., carbonate reduction.

The relative importance of some of these methylated substrates as the source for bacterial CH₄ is currently uncertain. However, CH₄ formation utilizing non-competitive substrates is known for environments such as rice paddies, salt marshes and other wetlands, i.e., those environments which are known to be major emission sources of CH₄ to the atmosphere (Cicerone and Oremland, 1988; Andreae and Schimel, 1989; Whiticar, 1990). Enhanced methanogenesis has also been obtained in culture experiments by additions of various ethylated and sulphide compounds (Oremland et al., 1988b) including diethylsulphide (DES), methanethiol (MeSH), ethanethiol (EtSH) and ethanol (EtOH). Their impact as substrates for methanogens is also currently unknown, but they are thought to be less important.

3. C and H isotope variations of methane in natural environments

The isotope data are reported in the standard δ-notation (e.g., δ¹³C, δD) expressed here in permil (%e):

\[ \delta_x = \left( \frac{R_x}{R_{x,\text{standard}}} - 1 \right) \times 10^3 \text{ (%e)} \]  

(4)
where $R_a$ is the $^{13}\text{C}/^{12}\text{C}$ or D/H ($=^{2}\text{H}^/H$) ratios relative to the PDB and SMOW standards, respectively.

The study of C and H isotope systematics based on in vivo observations of methanogenesis have, in the past, generally emphasized the competitive substrates. Whiticar et al. (1986) used the combination of C and H isotope data of CH$_4$, together with those of the coexisting bicarbonate and water species, to define compositional fields for the different methanogenic pathways. Using field measurements of CH$_4$ from various recent and ancient sedimentary environments, including marine, marsh, swamp and lacustrine settings, it was also possible with stable C and H isotopes to differentiate between the different bacterial and thermogenic CH$_4$ types. The combination of $\delta^{13}\text{C}_\text{CH}_4$ and $\delta\text{D}_\text{CH}_4$ values defining the various natural sources of CH$_4$ in the geosphere is demonstrated in the CD-diagram (Fig. 4). The atmospheric methane CD pair does not fall within any of the fields in Fig. 4 due to the isotopic offset caused by the C and H isotope effects associated with the photochemically mediated OH-abstraction reaction (e.g., Whiticar, 1993).

3.1. Thermogenic, non-bacterial, methane

Thermogenic CH$_4$ is generally, but not exclusively, enriched in $^{13}\text{C}$ compared with bacterial CH$_4$. The former has a $\delta^{13}\text{C}_\text{CH}_4$ range extending from roughly $-50\%e$ to $-20\%e$ (Fig. 4). The dissimilarity in isotope distributions of bacterial and thermogenic CH$_4$ accumulations is related to several factors including precursor compounds, the difference in type and magnitude of kinetic isotope effects (KIE) involved, and to the generally higher temperatures for
the thermogenic generation of hydrocarbons. Within
the range of $\delta^{13}C_{CH_4}$ values known for typical ther-
mogenic gases, it is possible to further classify them
according to the source rock type (kerogen type) and
maturity level from which they were derived. A
detailed discussion of isotope variations for thermo-
genic gases exceeds the framework of this paper (see
Schoell, 1988; Whiticar, 1994). However, in summary
the $\delta^{13}C_{CH_4}$ of thermogenic gases becomes
progressively enriched in $^{13}C$ with increasing matu-
re, eventually approaching the $^{13}C/^{12}C$ of the ori-
ignal organic matter or kerogen (and rarely even
heavier). In general, the carbon isotope separation
between thermogenic CH$_4$ and organic matter
($\delta^{13}C_{CH_4}-\delta^{13}C_{org}$) can vary from ca. 30‰ to 0‰.
Furthermore, hydrogen-rich organic matter, i.e.,
derived from $\delta^{13}C_{CH_4}$ values than, for example, coaly (type
III) kerogen sources. The hydrogen isotope ratios of
thermogenic CH$_4$ range from $\delta_2H_{CH_4}$ values of
approximately $-275‰$ to $-100‰$ (Fig. 4). Due to the
considerable overlap in $\delta_2H_{CH_4}$ between certain bac-
terial and thermogenic methane types, the hydrogen
isotope ratios of CH$_4$ are less useful as an isolated
parameter to classify natural gases. However, if they
are used in combination with additional molecular or
isotope compositional gas data, e.g., C$_2$-C$_4$, the
$\delta_2H_{CH_4}$ information can be particularly valuable. In
addition to thermogenic methane, the C- and H-is-
otope signatures and ranges of other non-bacterial
methanes, such as geothermal and hydrothermal, are
presented in Fig. 4 for comparison.

3.2. Bacterial methane

The term `bacterial’ is preferred over `biogenic’
because the carbon in both bacterial or thermogenic
natural gases, including most methane, is ultimately
derived from or has been part of the biological loop
of the exogenic carbon cycle.

Bacterial CH$_4$ has a wide range of C and H
isotope ratios (Fig. 4), varying in $\delta^{13}C_{CH_4}$ from
$-110‰$ to $-50‰$, and in $\delta_2H_{CH_4}$ from $-400‰$ to
$-150‰$. The bacterial fields in the CD-diagram
shown in Fig. 4 are delineated by compiled CH$_4$
data from natural environments (Whiticar et al.,
1986). Within this large range of values two primary
bacterial CH$_4$ fields were initially identified, namely,
methane that is formed in (1) freshwater and (2)
saline sedimentary environments. These fields are
now labeled in Fig. 4 as bacterial methyl-type fer-
tmentation and bacterial carbonate reduction, re-
spectively. Bacterial CH$_4$ accumulated in marine/saline
environments is generally more depleted in $^{13}C$ and
enriched in deuterium than observed in freshwater
environments (Fig. 4).

This initial distinction was based only on the
classification of the depositional environments and
did not explicitly consider the methanogenic path-
ways(s) involved. However, methanogenic path-
ways can also be inferred from the C and H isotope
data. This relies on the combination of environ-
mental information with the understanding of micro-
biological processes presented in Section 2, i.e.,
that carbonate reduction is the dominant
methanogenic pathway in marine environments and
methyl-type substrates (acetate) are more important
in freshwater environments.

The separation of the two bacterial CH$_4$ fields in
the CD-diagram can be made at approximate bound-
aries of $\delta^{13}C_{CH_4}$ of $-60‰$ and $\delta_2H_{CH_4}$ of $-250‰$
(Fig. 4). Although it is common to measure only the
carbon isotopes of CH$_4$, the hydrogen isotope ratios
of CH$_4$ are particularly helpful in defining the bacte-
rial CH$_4$ types. In certain instances, the CH$_4$
isotope data have a “transitional” isotope composition that
lies between the two fields. The explanation for this
overlap is related to the combined effects of: (1)
kinetic isotope fractionation by methanogens, (2)
mixtures of various pathways and/or CH$_4$
types (e.g., migration/diffusion) and (3) variations in C-
and H-isotope composition of precursor organic mat-
ter. These three explanations are expanded in Section 4.

4. Factors controlling isotope signatures in
bacterial methane

4.1. Kinetic isotope effects for carbon

The distribution of carbon isotopes during uptake
and metabolism of carbon compounds by metha-
nogens is controlled primarily by 1. isotope signa-
ture of source material and 2. KIEs. In the general
Table 1  
Magnitude of carbon isotope effects associated with methanogenesis

(A) Methanogenesis: natural observations

<table>
<thead>
<tr>
<th>Environment</th>
<th>Fractionation factor ($\varepsilon_C$) of carbon dioxide–methane</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freshwater (methylated compounds)</td>
<td>39 to 58</td>
</tr>
<tr>
<td>Marine (carbonate reduction)</td>
<td>49 to 95</td>
</tr>
</tbody>
</table>

(B) Methanogenesis: culture experiments

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Fractionation factors ($\varepsilon_C$)</th>
<th>Carbon dioxide–methane</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>68 to 77</td>
<td>40 to 54</td>
</tr>
<tr>
<td>Acetate</td>
<td>24 to 27</td>
<td>10</td>
</tr>
<tr>
<td>TMA</td>
<td>39</td>
<td>49</td>
</tr>
<tr>
<td>CO$_2$–H$_2$</td>
<td>55 to 58</td>
<td>55 to 58</td>
</tr>
<tr>
<td>DMS</td>
<td>44 to 54</td>
<td></td>
</tr>
<tr>
<td>DES</td>
<td>49</td>
<td></td>
</tr>
<tr>
<td>EtSH</td>
<td>54</td>
<td></td>
</tr>
</tbody>
</table>

case for each particular compound, the molecules with the lower isotopic mass (e.g., $^{12}$CH$_3$COOH as opposed to $^{13}$CH$_3$COOH) diffuse and react more rapidly and thus are utilized more frequently than the isotopically heavier species. This discrimination accounts for the strong depletion in $^{13}$C of bacterial CH$_4$ relative to the precursor substrates. The apparent magnitude of this isotope fractionation varies for different pathways (Table 1). For example, the fermentation of acetate involves an enrichment in $^{12}$C on the order of 25% to 35% for $\delta^{13}$C$_{CH_4}$ relative to $\delta^{13}$C$_{acetate}$. A significantly greater $^{12}$C enrichment (over 55% for $\delta^{13}$C$_{CH_4}$ relative to $\delta^{13}$C$_{carbon dioxide}$) is observed for methanogenesis by carbonate reduction.

In a closed system, the continued preferential removal of the isotopically lighter molecules from the carbon pool during methanogenesis results in a progressive shift in the residual substrate towards heavier, $^{13}$C-enriched values. Hence, there is a corresponding progressive $^{13}$C enrichment in CH$_4$ that is subsequently formed. This carbon isotope mass balance is commonly described by the distillation functions of Rayleigh (1896), that can be approximated by the general forms (Eqs. (5) and (6)) to describe the isotope ratio of the remaining reactant and accumulated product, respectively:

$$R_{t,i} = R_{i,i} f^{(\alpha - 1)}$$  \hspace{1cm} (5)

and

$$R_{p,t} = R_{t,i} \frac{(1 - f^\alpha)}{(1 - f)}$$  \hspace{1cm} (6)

where $R_{t,i}$ is the isotope ratio of the precursor substrate, e.g., acetate or CO$_2$, and accumulating initially ($i$) and at time $t$, and $R_{p,t}$, is the isotope ratio of the accumulating product, e.g., methane, $f$ is the fraction of initial substrate remaining at time $t$ and $\alpha$ is the kinetic isotope fractionation factor.

The isotope separation in $\delta$-notation between two compounds, e.g., $\delta^{12}$C$_{CO_2}$ and $\delta^{13}$C$_{CH_4}$, can be expressed as the isotope separation factor ($\varepsilon_C$):

$$\varepsilon_C \approx 10^3 \ln \alpha_C = 10^3 (\alpha_C - 1)$$  \hspace{1cm} (7)

Note that the approximations in Eq. (7) are valid for relatively small isotope separations, typically less than 25% between species. For larger separations, common with hydrogen isotopes, these assumptions can lead to significant errors. In these cases, the fractionation factor $\alpha_C$ is better defined as the ratio of the reaction rate constants for the two isotopes, e.g., referring to Eq. (1), $\alpha_C = \frac{k_{^{13}k}}{^{12}k}$, where:

$$^{12}\text{CO}_2 \rightarrow ^{12}\text{CH}_4 \hspace{1cm} \text{and} \hspace{1cm} ^{13}\text{CO}_2 \rightarrow ^{13}\text{CH}_4.$$  \hspace{1cm} (8)
For equilibrium isotope situations, the relationship between $\alpha$ and $\delta$ is defined as:

$$\alpha_{A-B} = \left( \frac{\delta_A + 10^3}{\delta_B + 10^3} \right). \quad (9)$$

Normally, isotope equilibrium cannot be assumed for these kinetic reactions. However, this notation has sometimes been used to express the magnitude of the isotope partitioning. These Rayleigh distillation functions expressions (Eqs. (5) and (6)) can be converted to $\delta$-notation and simplified by the substitution with $\varepsilon$ (Eq. (7)) and taking $F = 1 - f$.

$$\delta^{13}C_{\text{substrate},i} = \delta^{13}C_{\text{substrate},i} + \varepsilon \ln(1 - F), \quad (10)$$

and for the instantaneous product formed:

$$\delta^{13}C_{\text{methane},i} = \delta^{13}C_{\text{substrate},i} + \varepsilon (1 + \ln(1 - F)). \quad (11)$$

The actual location(s), e.g., at the membrane and/or enzymatic level, where the isotope fractionation occurs during methanogenesis is still uncertain. However, the consequence of this fractionation is that within a specific environment a considerable

Fig. 5. Theoretical shifts in $\delta^{13}C_{\text{CH}_4}$ and $\deltaD_{\text{CH}_4}$ as a result of isotope variations in original organic matter and through secondary effects (substrate depletion and methane oxidation).
range in carbon isotope values can be observed which is dependent on the degree to which the substrate has been depleted (Fig. 5). In cases of extensive substrate exhaustion, the carbon isotope values of bacterial CH4 can approach those of the original organic matter. In some instances where mass balance is not maintained, such as loss of the methane initially formed, the methane can in fact be more enriched in 13C than the starting precursor material. Although such variations in the δ13C(CH4) values exist, they are most commonly observed in methanogenic culture experiments.

4.2. Temperature effects on carbon isotope values

The effect of temperature on the fractionation of carbon isotopes associated with methanogenesis is poorly understood. Conrad and Schütz (1988) demonstrated a 2.5- to 3.5-fold increase in CH4 generation due to a 10°C temperature rise, but it is not known if this would affect the KIE. Later, they reported that the temperature could influence the rates and pathways of methanogenesis (Schütz and Conrad, 1996; Schütz et al., 1997). Whiticar et al. (1986) showed that there is a general trend to lower fractionations with increasing temperature. Empirically, one could expect a decrease in the isotope effect at higher temperatures. A rough, albeit incomplete, test is provided by comparing the a13C between CO2 and CH4 from carbonate reducing methanogenesis at various temperatures (Table 2). This relationship, shown in Fig. 6, indicates, as expected from thermodynamic considerations, that the KIE decreases significantly with increasing temperature. Over a 110°C temperature rise, the isotopic partition of δ13C(CO2)−δ13C(CH4) decreases from 100±ε to 40±ε, equivalent to an εc change of ca. 86 to 39 for CO2−CH4 pair (Eq. (7)). Botz et al. (1996) also documented a temperature dependence of isotope fractionation during biological methanogenesis. Their culture experiments of methanogenesis by carbonate reduction with Methanococcales at 35–85°C gave εc values of 76 to 47, respectively.

In addition to temperature, it has been suggested that the carbon isotope fractionation is dependent on other factors such as substrate concentration and rates of methanogenesis. Zyakun (1992) reported for culture experiments that the magnitude of carbon isotope fractionation during methanogenesis by carbonate reduction was dependent on the CO2 gassing rate. Above a critical CO2 threshold the CO2−CH4 isotope separation was constant, but decreased dramatically as the amount of CO2 available dropped. Fuchs et al. (1979) demonstrated a similar result. In addition, Zyakun (1992) stated that the magnitude of the carbon isotope fractionation is dependent on the rate of methanogenesis and the type of methanogens and substrates (CO2 and methylated). It is clear from these experiments that the isotope separation between the precursor and methane decreases with the degree of substrate utilization. However, the magnitude of separation is unlikely to be caused by a change in the isotope effect, i.e., a change in the enzymatic kinetic reaction rates for 13C vs. 12C (Eq. (8)). It is more likely that the decrease in precursor-methane isotope separation is due to a ‘reservoir effect’ caused by substrate depletion and/or diminished rates of substrate transport across the membrane walls of the methanogens. Thus, the maximum expression of the carbon isotope effect corresponds to the situations with high substrate levels. Increased levels of methanogenesis at higher temperatures is well documented. For example, Martens et al. (1986) and Jedrysek (1995) showed that diurnal variations in temperature could influence the carbon isotopes, but again this is probably a methanogenic rate effect and not enzymatic. Iversen

<table>
<thead>
<tr>
<th>Location/bacterium</th>
<th>Temperature (°C)</th>
<th>εc CO2−CH4</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bransfield Strait (Antarctic)</td>
<td>~ 1.3</td>
<td>86 to 95</td>
<td>Whiticar and Suess (1990)</td>
</tr>
<tr>
<td>Methanosarcina barkeri</td>
<td>20</td>
<td>77</td>
<td>Whiticar, Müller, Blaut (unpublished data)</td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>58</td>
<td></td>
</tr>
<tr>
<td>Thermoautotrophicum</td>
<td>65</td>
<td>34 to 40</td>
<td>Fuchs et al. (1979)</td>
</tr>
<tr>
<td>Hyperthermophil</td>
<td>110</td>
<td>40</td>
<td>Whiticar, Stetter, Huber (unpublished data)</td>
</tr>
</tbody>
</table>

Table 2: Effect of temperature on the magnitude of carbon isotope effects associated with methanogenesis by carbonate reduction.
4.3. Hydrogen isotope effects

Our understanding of the hydrogen isotope effects associated with methanogenesis is limited. Empirical evidence suggests that substantial enrichments in $^1$H do occur as hydrogen is transferred to the methane molecule from either organic precursors or formation water. This hydrogen isotope partitioning is present regardless of the methanogenic pathway, but the magnitude may be dependent on it. (Whiticar et al., 1986; Burke et al., 1988a). As expected by the larger mass difference between $^1$H and $^3$H than $^{12}$C vs. $^{13}$C, the hydrogen isotope partitioning is larger than carbon isotopes. As discussed below, it remains unresolved to what extent kinetic vs. equilibrium mechanisms dominate the hydrogen isotope distributions in methane. In addition, there are suggestions that factors such as hydrogen concentration (Burke, 1992, 1993) or hydrogen isotope exchange (de Graaf et al., 1996) complicate the situation.

4.4. Mixtures

Bacterial CH$_4$ with “mixed” or “transitional” isotope signatures can often be present in a gas sample. This could be the result of contributions of CH$_4$ from different methanogenic pathways (Fig. 5) which may be operating in the same location, either concurrently or successively. For example, the form of methanogenesis can shift from one substrate to another, such as the succession to carbonate reduction after the acetate pool is exhausted. This is observed in some freshwater systems (e.g., Hornibrook et al., 1997). Work in shallow marine sediments by Sansone and Martens (1981), Martens et al. (1986), Burke et al. (1988b) and Kelley et al. (1992) demonstrated that shifts to different methanogenic substrates or isotope signatures might also be controlled by seasonal conditions.

These gas mixtures could also be the result of physical mixing of different CH$_4$ pools in response to vertical or lateral migration or diffusion of gas. This mixing is not necessarily restricted to bacterial gas, but can also involve admixtures of thermogenic gas.

4.5. Isotope variations in precursor organic matter

It is more difficult to assess the third point, namely, variations in isotope composition of the precursor organic matter. C and H isotope values of bulk organic matter that are available for specific environments exhibit the approximate isotope ranges shown in Fig. 5. With the exception of dissolved $\Sigma$CO$_2$ (see below), isotope measurements are sparse for specific methanogenic substrates in natural environments. The magnitude to which the C and H isotope compositions of the various methanogenic substrates deviate from those of the bulk organic matter is not well known, nor is the magnitude of any intramolecular isotope fractionation present in
such substrates well understood (Yankwich and Promislov, 1953; Meinschein et al., 1974; Rossmann et al., 1991). However, it has been assumed up to now that the natural C and H isotope signatures of a substrate in a specific setting are similar to those of the bulk organic matter, provided that severe substrate depletion has not occurred and that large inter- or intramolecular variations are not present. Analytical techniques now are available to measure C and H isotope ratios in methane and the precursors in the picomole range (e.g., Whiticar et al., 1998a,b), that should help resolve this issue.

Methylated substrates available for fermentation by methanogens often are present only in minor amounts. This is due to an effective combination of formation and utilization that leads to a relatively short residence time. Although this may control the rate of methanogenesis via methyl-type fermentation,
the amount of methane produced can exceed saturation (Fig. 3). The low concentration of methanogenic substrates, such as organic acids, makes it difficult to isotopically model their removal (e.g., Sansone and Martens, 1981; Blair and Carter, 1992). In contrast, organic-rich marine sediments can have substantial bicarbonate concentrations (> 100 mM). These amounts permit more readily the modeling of carbon isotope systematics for methanogenesis by carbonate reduction. Claypool (1974) provided one of the first models using gas and interstitial data from sediment cores of the Deep Sea Drilling Program (DSDP, later Ocean Drilling Program, ODP). An illustration of results similar to those found in numerous DSDP, ODP and other sediment cores is illustrated by the 7.5-m piston core taken at station 1327 in the Bransfield Strait, Antarctic (Whiticar and Suess, 1990). In this case, CH₄ concentrations first exceed 100 ppb (weight of gas/weight of wet sediment) beneath the sulphate reduction zone (Fig. 7a). The continual removal of dissolved bicarbonate by methanogens is marked by the reduction in the increase of dissolved ΣCO₂ concentration with depth from the sulphate reduction zone to the zone of methanogenesis. This change was accompanied by a sharp decrease in molar C:N ratio (carbonate alkalinity:ammonia) due to the preferential uptake of carbon over nitrogen. The more rapid uptake and utilization of 12 C bicarbonate by methanogens in core 1327 leads to a gradual shift in δ³¹C CO₂ towards heavier values in the older sediments (Fig. 7b). This depletion is in turn expressed in the δ³¹C of the CH₄, which also becomes heavier with sediment depth in the methanogenic zone as predicted by the Rayleigh function (Eq. (15)). The carbon isotope separation between CO₂ and CH₄ in the CH₄ zone of core 1327 is consistently around 80%e. The fact that the CO₂ concentration does not substantially decrease in this zone, indicates that CO₂ is continually added by organic remineralization to the pool in the methanogenic zone, although overall there is a 12 C depletion by the methanogens. This strict relationship between δ³¹C CO₂ and δ³¹C CH₄ provides important information about methanogenic pathways, as is developed in greater detail below.

The variations in inter- and intramolecular isotope distributions and effects for hydrogen in organic matter are much less well constrained than for carbon. Typically only determinations of bulk δDCH₄ are available for natural environments without the respective D/H values for specific substrates or hydrogen isotope distributions within the precursor molecules. Some culture experiments have used additions of water and substrates with known hydrogen isotope values to assess the methanogenic processes and magnitudes of the related isotope effects.

5. Isotope systematics of methanogenesis with the coexisting CO₂ and water

5.1. Carbon isotopes

Although the CD-diagram (Fig. 4) can be used to differentiate the major CH₄ sources, the various secondary effects discussed above can obscure the signature of source(s) under certain conditions. The ambiguity in determining the methanogenic pathway can sometimes be resolved by considering the isotope information of CO₂ (δ¹³C CO₂) and formation water (δD H₂O) which coexist with the CH₄, e.g., in the corresponding pore fluid or water mass.

In the case of carbon, the isotope separation factor between δ¹³C CO₂ and δ¹³C CH₄ (εc) is defined by Eq. (7). In natural marine or saline environments, εc associated with methanogenesis predominantly by carbonate reduction ranges from 49 to over 100, with values most commonly around 65 to 75 (Fig. 8, after Whiticar et al., 1986). In comparison, the corresponding fractionation factors for methanogenesis in freshwater environments, i.e., those dominated by fermentation of methylated substrates, are distinctly lower with εc values typically ranging between 40 and 55 (Fig. 8). A key point is that this carbon isotope fractionation factor tends to remain more or less constant for each specific setting or diagenetic environment. Thus, although the δ¹³C CH₄ can shift dramatically in response to the changing isotope ratio of the depleting substrate, the isotope separation between CH₄ and CO₂ remains consistent for, and indicative of, the particular methanogenic pathway. This is illustrated in Fig. 8 by the production arrow. Similarly, the environments where methanogenesis operates with mixed substrates can also be recognized using the CH₄–CO₂ pairs despite large variations in the actual δ¹³C CH₄ values.
The carbon isotope fractionation factors between CO$_2$ and CH$_4$, associated with methanogenesis in marine and freshwater environments, are summarized in Table 1. For comparison, Table 1 also provides the carbon fractionation factors between CH$_4$ and various substrates (after Oremland et al., 1988a,b; Whiticar, 1994), as determined by culture experiments. Similar to that reported by Rosenfeld and Silvermann (1959) and Krzycki et al. (1987), methanol exhibits the largest carbon isotope fractionation between a methylated substrate and the methane formed, i.e., $\varepsilon_{C_{\text{methanol-methane}}} = 68$ to 77 (Table 1). Predictably, the largest CO$_2$–CH$_4$ separation was observed for CO$_2$-reduction, $\varepsilon_C = 58$ (Table 1). This is comparable to the carbon isotope fractionation value of $\varepsilon_C$ ca. 57 reported by Zyakun (1992) for carbonate reduction culture with CO$_2$ concentration as above and independent of the critical threshold. Similarly, Waldron et al. (1998) found $\varepsilon_C$ to be ca. 55 for carbonate reduction in their batch cultures. In the same paper, the authors reported $\varepsilon_C$ to be ca. 24 for acetate fermentation.

5.2. Hydrogen isotopes

The relationship between the hydrogen isotope ratios of bacterial CH$_4$ and the coexisting formation water can also be employed to track methanogenic pathways. This consideration, introduced by Nakai et al. (1974) and developed by Schoell (1980), Woltmate et al. (1984) and Whiticar et al. (1986), is due to methanogens deriving a specific proportion of their hydrogen for CH$_4$ formation ultimately from the water in which they live. For carbonate reduction, the coexisting formation water is essentially the initial hydrogen source (100%). This has been confirmed experimentally by Pine and Barker (1956), Fuchs et al. (1979) and Daniels et al. (1980). The fermentation of methylated substrates is thought to involve an intact transfer of the three methyl hydro-
gens (75%) to the CH$_4$ molecule (Fig. 9). This intact hydrogen transfer has been demonstrated in culture studies with deuterated substrates (Pine and Barker, 1956; Pine and Vishniac, 1957; Daniels et al., 1980; Whiticar et al., 1998a,b).

The empirical relationship expected between $\delta$D$_{CH_4}$ and the coexisting $\delta$D$_{H_2O}$ as depicted in Fig. 9 should be:

$$\delta D_{CH_4} = m(\delta D_{H_2O}) - \beta,$$

where $m = 1$ holds for carbonate reduction, and $m = 0.25$ is expected for the fermentation of methylated substrates. The values for $\beta$ in Eq. (12) are dependent on the isotope effects involved in the abstraction and transfer of the hydrogen. For the methylated compounds, they are also additionally dependent on the hydrogen isotope ratio of the methyl hydrogens. The combined hydrogen isotope effects during carbonate reduction in various environments appear to be remarkably consistent around 160 to 180 for CH$_4$ relative to the formation water, as shown in Fig. 10.

There is probably not a universal value for $\beta$ with respect to methylated substrates. Rather, the magnitude of the isotope offset is site specific. However, most studies suggest that $\beta$ associated with methanogenesis by methyl-type fermentation is significantly larger than for carbonate reduction. Based on the current SEOS (School of Earth and Ocean Sciences, Victoria) and BGR (Bundesanstalt für Geowissenschaften und Rohstoffe, Hannover) data bases, values of $\beta$ known for methylated substrates are greater than 300 and extend up to 377.

In methanogenic environments with more than one active pathway, the situation is more complicated. To accommodate the mixture of two pathways, e.g., combination of carbonate reduction and fermentation of methylated substrates, in the isotope mass balance, Eq. (7) can be modified to:

$$\delta D_{CH_4} = f(\delta D_{H_2O} - 160)$$

$$+ (1 - f)\left[(0.75\delta D_{methyl})
+ (0.25\delta D_{hydrogen})\right],$$

where $f$ is the relative proportion of methanogenesis by carbonate reduction ($f = 0$ to 1) to fermentation by methylated substrates, $\delta$D$_{methyl}$ is the hydrogen isotope ratios of the intact-transferred methyl group and $\delta$D$_{hydrogen}$ is that of the final hydrogen incorporated. $\delta$D$_{CH_4}$ and $\delta$D$_{H_2O}$ are readily measured, but our present deficiencies in precise information on the distribution of hydrogen isotopes in methylated substances make solution of Eq. (13) difficult. However, ranges on the parameters can be estimated.

In recent years, the hydrogen isotope systematics associated with methanogenesis have been revisited. Sugimoto and Wada (1993, 1995) investigated the relationships between $\delta$D$_{CH_4}$ and $\delta$D$_{H_2O}$ for rice paddy soil during methanogenesis in culture experiments. Although they only indirectly calculated the relative importance of the two methanogenic pathways, the authors reported values for $m$ and $\beta$ according to Eq. (12) of 0.437 and 302 (L4 in Fig. 10), respectively, for methyl-type fermentation. Interestingly, they also calculated $m$ and $\beta$ values of 0.683 and 317 for the carbonate reduction pathway (L6 in Fig. 10). These latter culture values contrast strongly with those known empirically for natural
Fig. 10. Dependence of hydrogen isotope ratio in methane ($\delta_{\text{CH}_4}$) as a function of the hydrogen isotope ratio of the coexisting formation water (e.g., porewater, water column) after Eqs. (12) and (13). Methanogenesis by carbonate reduction follows a slope ‘‘m’’ of 1 with an isotope offset ‘‘b’’ of ca. 160‰ ($L_1$, $F$:$R = 0$:$100$). The methyl-type fermentation pathway has a slope $m$ of ca. 0.25 and an offset $b$ of 300‰ to 370‰ ($L_2$, $F$:$R = 100$:$0$). $L_3$ shows the typical $(\delta D_{\text{H}_2\text{O}} - \delta D_{\text{CH}_4})$ relationship observed by Whiticar et al. (1986) for freshwater ($F$:$R = 80$:$20$). The relationships of Sugimoto and Wada (1995) for methyl-type fermentation ($L_4$) and carbonate reduction ($L_6$) and of Waldron et al. (1998) for a mixture of the two pathways ($L_5$) are shown as dashed lines. Additional data of Balabane et al. (1987), Burke et al. (1988a), and Grossman et al. (1989) are shown as points 7–9.

environments. The explanation offered by Sugimoto and Wada (1995) for the difference in the carbonate reduction values between freshwater and marine environments was that the former has higher hydrogen concentration and longer hydrogen residence time. A similar effect was proposed by Burke (1993). Hornbrook et al. (1997) reported that their natural wetland studies fit the model of Whiticar et al. (1986). They countered that in comparison with natural settings, the high H$_2$ concentrations in the culture experiments of Sugimoto and Wada (1995) may have affected the magnitude of the hydrogen isotope partitioning and led to the observed discrepancies. Burke et al. (1988a) and Grossman et al. (1989) found $(\delta D_{\text{H}_2\text{O}} - \delta D_{\text{CH}_4})$ relationships indicating mixed reactions (Fig. 10, points 7 and 8, respectively) whereas the culture study of Balabane et al. (1987) gave $(\delta D_{\text{H}_2\text{O}} - \delta D_{\text{CH}_4})$ values similar to methyl-type fermentation (Fig. 10, point 9).

Sugimoto and Wada (1995) suggested that hydrogen isotope exchange could explain the difference in $m$ and $b$ values (Eq. (12)) for methyl fermentation between their results (0.437 and 302) and those in (Whiticar et al., 1986) (0.25, 321). Support for hydrogen isotope exchange during acetate fermentation comes from the work of de Graaf et al. (1996). Certainly some of the methanes strongly depleted in deuterium, i.e., those lighter than $\delta D_{\text{CH}_4}$, ca. $-400‰$, are difficult to explain by direct methyl hydrogen incorporation. For example, if one assumes $\delta D_{\text{eq}}$ value of $-120‰$ for the three methyl hydrogens donated, and a $\delta D_{\text{CH}_4}$ of $-400‰$, this would
give by Eq. (13) a non-sensical $\delta D$ value of $-1240\%e$ for the final hydrogen (Fig. 11). This indicates that either the hydrogen in the precursor methyl group is more depleted in deuterium than bulk organic matter or some isotopic exchange has occurred.

Waldron et al. (1988) suggested that the hydrogen isotope signature in bacterial methane is set by the enzymatic incorporation of hydrogen from water and not by the precursor hydrogen on the methyl group. They proposed that this enzymatic control is operative for both acetoclastic and carbonate reduction methanogenic pathways. They calculated the magnitude of this isotope fractionation ($\alpha_{H_2,0\rightarrow CH_4}$) to be 1.47. Waldron et al. (1988) found values for $m$ and $b$ (Eq. (12)) of 0.623 and 319 for cultures of landfill material with both methanogenic pathways operative at unknown relative proportions (L5 in Fig. 10).

The processes controlling the distribution of hydrogen in methane during methanogenesis remain unclear. Certainly, numerous questions require detailed attention, including that of possible hydrogen isotope exchange, effects of hydrogen partial pressures, rates of methanogenesis, and the translation of results from culture experiments to natural environments.

6. Isotope effects associated with bacterial methane oxidation

Bacterial consumption is a major sink of CH$_4$ in the geosphere and in water columns. Without this hydrocarbon metabolism, the flux of CH$_4$ into the atmosphere could be orders of magnitude greater, and its impact on the global atmospheric thermal and chemical budgets would be even more significant (Whiticar, 1993). Bacterial CH$_4$ consumption can be by either aerobic or anaerobic oxidation. The former is a common process in freshwater settings normally at the anoxic/oxic interface, for example within soils or within the uppermost centimeters of sediments in swamps or lakes (e.g., Rudd and Hamilton, 1975; Bossard, 1981; Conrad, 1989; Steudler et al., 1989). Anaerobic CH$_4$ consumption was controversial for a long period amongst microbial ecologists, but the arguments presented for anaerobic oxidation from a geochemical perspective are convincing (Iversen and Blackburn, 1981; Iversen and Jørgensen, 1985). This methanotrophic process is particularly active in marine and brackish sediments, normally at the base of the sulphate reduction zone. The strongest geochemical indications for anaerobic CH$_4$ oxidation are: (1) the dramatic decrease in CH$_4$ concentrations across the CH$_4$ production–sulphate reduction boundary (Fig. 3), (2) the preferential loss of CH$_4$ compared with higher molecular weight hydrocarbons in the same location, and (3) the systematic shift in C and H isotope ratios of CH$_4$ consistent with the loss of CH$_4$ in the consumption zone (e.g., Whiticar and Faber, 1986; Alperin et al., 1988).

Over the past 20 years studies of CH$_4$ distributions in marine sediments identified the diagenetic zonation between sulphate reduction and CH$_4$ formation (e.g., Claypool and Kaplan, 1974; Martens and Berner, 1977; Whiticar, 1982). In the simplest of terms, the concave downward shape of the depth
profile of dissolved sulphate concentration (Figs. 3 and 7a) is the result of the balance between downward diffusing sulphate, microbial uptake by SRB and vertical pore fluid advection. Analogously, the concave upward shape of the CH$_4$ concentration profile could not be maintained by diffusion and advection mechanisms alone (see Reeburgh, 1976; Martens and Berner, 1977). Bacterial anaerobic oxidation at the base of the sulphate zone is responsible for active CH$_4$ removal. However, complete consumption of CH$_4$ does not occur and trace levels of CH$_4$ are found in both sulphate-bearing and oxic environments. Whiticar and Faber (1986) and other investigators, including Martens and Berner (1977), Oremland et al. (1987) and Adams and van Eck (1988), demonstrated that CH$_4$ can persist in the presence of trace amounts of sulphate, approximately 0.2 mM in the sulphate zone of marine, brackish and freshwater sediments. Possible explanations for this are: (1) restricted in situ methanogenesis, (2) in these environments there is a CH$_4$ concentration threshold below which CH$_4$ is not attractive to methanotrophs, or (3) that specific nutrients or co-metabolites are not present. Methane buildup in the sulphate zone may be related to consumption rates being lower than the influx of CH$_4$. Vertical gas ebullition could rapidly introduce CH$_4$ into the sulphate zone, or in certain circumstances the buildup could be due to inhibition of CH$_4$ oxidation.

Bacterial consumption of CH$_4$ appears to proceed at a significantly greater rate than for higher molecular weight hydrocarbon gases such as ethane, propane.

Fig. 12. Natural gas interpretative (‘‘Bernard’’) diagram after Bernard et al., 1978 combining the molecular and isotope compositional information. Lines $\lambda$ and $\lambda'$ are calculated mixing lines for possible gas bacterial and thermogenic mixtures with end-member isotope ($\delta^{13}$C$_{CH}_4$) and molecular ($C_1/(C_2 + C_3)$) compositions of $-100\%$, $10^5$; $-45\%$, $2(\lambda)$ and $-55\%$, $5000$; $-45\%$, $50 (\lambda')$, respectively. The relative compositional effects of migration or oxidation are also indicated.
or butanes. Thus, as CH₄ is continually removed, a molecular fractionation occurs which relatively enriches the residual hydrocarbon gas in the higher homologues. The proportion of CH₄ in a hydrocarbon gas mixture can be expressed on a volume percentage basis by the “Bernard parameter”, C₁/(C₂ + C₃) (see Bernard et al., 1978; Whiticar, 1990). In CH₄ formation zones which are devoid of thermogenic hydrocarbons, C₁/(C₂ + C₃) is typically 10⁻³ to 10⁻². In the CH₄ consumption zone the C₁/(C₂ + C₃) ratio can decrease to values of less than 10. Molecular ratios less than 50 are also typical for thermogenic hydrocarbon gases, as shown in Fig. 12 so that CH₄ oxidation can lead to difficulties in the interpretation of natural gas sources.

Analogous to methanogenesis, the bacterial uptake of CH₄ is associated with a KIE that enriches the residual CH₄ in the heavier isotope. This fractionation is most pronounced for the CH₄ carbon isotopes, and in some cases, has been observed for the hydrogen isotopes in CH₄. The magnitude of the carbon KIE during CH₄ consumption is much lower than that associated with substrate uptake by methanogens. Culture experiments and models have been used to substantiate this (compare Tables 1 and 3).

Several investigators have demonstrated with aerobic culture studies that¹²C±CH₄ is preferentially removed during its oxidative consumption. The magnitude of the carbon isotope fractionation (εc) in culture experiments varies between 5 and 30 (Table 3). The only determinations of the hydrogen isotope fractionation factor for CH₄ oxidation (εH) in an aerobic culture study lay between 95 and 285 (Coleman et al., 1981).

Three models were developed by Whiticar and Faber (1986) to calculate the carbon isotope fractionation of CH₄ during its anaerobic consumption. These models are termed: (1) residual methane, (2) C₂+ enrichment and (3) methane–carbon dioxide partition models. A fourth, a concentration gradient model, was published by Alperin et al. (1988). The latter were also able to model the hydrogen KIEs for CH₄ oxidation at the same location.

The first model (residual methane) uses the relationship of δ¹³C(CH₄) to the CH₄ concentration in interstitial fluids, as CH₄ is removed by bacterial oxidation the residual gas is continually enriched in the heavier carbon isotope fraction (Fig. 13). The fractionated CH₄ uptake is described by a closed-system Rayleigh function similar to Eq. (5):

\[
δ^{13}C_{CH_4} = \frac{δ^{13}C_{CH_4,t} + ε \ln(1 - F)}{C_{CH_4,t}}
\]

where \(δ^{13}C_{CH_4,t}\) is the carbon isotope ratio for the initial methane pool prior to oxidation (e.g., in the main CH₄ formation zone), \(δ^{13}C_{CH_4,t}\) is that at time \(t\). To account for variations of the initial CH₄ concentrations between different environments, the initial CH₄ concentration pool is normalized to 100%. The shift in \(δ^{13}C_{CH_4}\) to more positive values as a function of CH₄ consumption is shown in Fig. 14. The best fit to the data was obtained with a carbon fractionation factor (εc) of about 4. Larger values of εc around 20, chosen to model the data, do not describe the system satisfactorily.

The second model (C₂+ enrichment) uses the simultaneous change in both the molecular (B = C₁/(C₂ + C₃)) and isotope composition (\(δC = δ^{13}C_{CH_4}\)) of the bacterial gas during CH₄ consump-

![Table 3](image-url)
tion to calculate the isotope fractionation ($\varepsilon_C$) according to

$$
\varepsilon_C = \frac{\ln B_t - \ln B_i}{(\ln B_t - \ln B_i)/10^3 + (\delta C_t - \delta C_i)} + 10^3
$$

(15)

where the subscripts $i$ and $t$ are for the initial and temporal conditions, respectively (see Whiticar and Faber (1986) for derivation of Eq. (10)). The compositional shifts for a variety of marine, marsh and brackish sediments are shown in Fig. 15 (a modified version of Fig. 12). The carbon isotope fractionation factors vary from $\varepsilon_C = 7$ to $14$, as indicated in the figure. Other data from the Antarctic (Whiticar and Suess, 1990) gave lower $\varepsilon_C$ values between 1.8 and 5. The explanation for the lower bacterial selectivity for these samples is not clear, but it may be related to the cold sediment temperatures ($-1.4^\circ$C).

As described in Section 5, the carbon isotope separation between CH$_4$ and CO$_2$ (Eq. (7)) is fairly constant and indicative of the formation pathway (methane–carbon dioxide partition model). In contrast, CH$_4$ oxidation causes a clear, strong decrease in the carbon isotope separation ($\delta^{13}$C$_{CO_2}$–$\delta^{13}$C$_{CH_4}$), as indicated in Fig. 8. Initially, the greater amounts of CO$_2$ than CH$_4$ typically present obscure the oxidation trend. However, towards the latter stages of consumption, the carbon isotope separation is observed to have $\varepsilon_C$ values between $5$ and $25$. From the $\delta^{13}$C$_{CO_2}$–$\delta^{13}$C$_{CH_4}$ coexisting pairs it is not only possible to distinguish between the methanogenic pathways but also to evaluate CH$_4$ consumption (Fig. 8).

Alperin et al. (1988) determined the isotope shifts in CH$_4$ from anaerobic Skan Bay sediments as a function of the CH$_4$ concentration gradient (concentration gradient model). By subtracting the possible effects of isotope fractionation associated with diffusion, they were able to apply diagenetic equations to estimate the magnitude of both the C and H isotope effects for anaerobic CH$_4$ oxidation. The carbon isotope fractionation factor $\varepsilon_C$ was found to be $8$. The hydrogen isotope fractionation factor ($\varepsilon_D$) for the same samples was $146$, which is consistent...
with the values reported for aerobic cultures by Coleman et al. (1981).

The possibility of aerobic methane consumption in soils was recognized by Keller et al. (1983) and Seiler et al. (1984), but only more recently it has been accepted as an important sink of atmospheric methane (e.g., Steudler et al., 1989; Ojima et al., 1993). Steudler et al. (1989), Mosier et al. (1991) and others have indicated that there is a close relationship between methanotrophs and ammonia-oxidizing bacteria in soils. The carbon KIEs associated with the soil uptake of methane have been reported by King et al. (1989), Tyler et al. (1994) and Steudler and Whiticar (1998) (Table 3). The former two, using chambers and incubations, respectively, found $\epsilon_{C_{\text{CO}_{2}}}^{CH_{4}}$ values around 16 to 27.

The latter, using in situ gas microsampling of soil gases at Harvard Forest found carbon isotope effects for the consumption to be significantly lower than 5%. The range of C and H isotope effects from experimental and field observations are summarized in Table 3. Overall, it can be commented that carbon isotope fractionation during CH$_4$ oxidation in anaerobic sediments is less than in aerobic culture studies. In addition, methanotrophs appear to be less selective between the lighter and heavier carbon isotopes than the methanogens.

7. Conclusions

In combination, the C and H isotope signatures of CH$_4$ are frequently adequate to reliably characterize bacterial or thermogenic natural gas types. Situations, such as mixing of different natural gases or where extreme substrate depletion and consumption occur, could produce ambiguous methane isotope signals. In these cases, the C- and H-isotopes of methane, in concert with the coexisting isotope information on CO$_2$ and H$_2$O, are excellent tracers of the processes of bacterial CH$_4$ formation and consumption. The magnitudes of the KIEs are consistent and sufficiently large to generate significant isotope fractionations that are diagnostic for the various processes. A clear understanding of these effects is necessary to relate the isotope signal of a natural gas to its source.

In view of the increasing atmospheric CH$_4$ concentration (ca. 0.5%–1% annually in the lower troposphere) and, as a radiatively active atmospheric trace gas having direct effect on global climate change, it is important to attach precise isotope signatures to the various CH$_4$ sources. Stable isotopes are an elegant method to differentiate the various fluxes and numerically constrain the magnitudes of both CH$_4$ emissions to and removal from the lower troposphere. In this way our comprehension of geochemical and microbiological processes involved in natural gas habitats and compositions within the geosphere are the key to understanding atmospheric CH$_4$ change.

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References


Iversen, N., Blackburn, T.H., 1981. Seasonal rates of methane


