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Isotope fractionation in the systems CH_4–H_2O and CH_4–CO_2 during microbial methane genesis in the Earth's crust

L.K. Gutsalo

Institute of Physical Chemistry of the Ukrainian National Academy of Sciences, 3a ul. Nauchnaya, Lvov, 79053, Ukraine

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Abstract

Distribution of hydrogen and carbon isotopes (D/H and $^{13}C/^{12}C$) in the microbial systems CH_4–H_2O and CH_4–CO_2 was studied in different world's regions. According to the distribution of these isotopes in the above systems, two types of microbial methane are recognized in the Earth's crust: (1) resulting from CO_2 reduction and (2) produced through acetate fermentation. In the case of methane 1, the biologic distribution of hydrogen and carbon isotopes in the above systems corresponds to the thermodynamic isotope exchange equilibrium at a given temperature of the methane genesis medium. In the case of methane 2, the same systems show mainly a nonequilibrium distribution of these isotopes. We have revealed a linear relationship between the isotopic compositions of CH_4, H_2O, and CO_2: $\delta D(CH_4) = \alpha^b_D D\delta D(H_2O) + b$ and $\delta^{13}C(CH_4) = \alpha^b_C C\delta^{13}C(CO_2) + d$, where α^b_D and α^b_C are the general factors of biologic nonequilibrium fractionation of hydrogen and carbon isotopes, respectively, in the systems CH_4–H_2O and CH_4–CO_2. These factors are determined from the equations $10^3 \ln \alpha^b_D = -477.357(10^6/T^2) + 3458.55$ and $10^3 \ln \alpha^b_C = -277.954(10^6/T^2) + 1988.677$, where T(K) is the temperature of the acetate methanogenic medium. The values of α^b_D and α^b_C do not depend (in contrast to the values of b and d) on the kind of bacteria and the temperature of the methane genesis medium. Based on thermodynamic data, we proposed a model for the formation of the isotopic composition of microbial methane in nature. Variations in the hydrogen and carbon isotope compositions of microbial methane in various geologic objects are due mainly to the variations in the temperature of the methanogenic medium and the mixing (in different proportions) of methane 1 with methane 2. The portions of acetate fermentation methane in the total balance of microbial methane in different geologic objects vary over a wide range of values: 52 to 100% in marine deposits of Cape Lookout Bight, North Carolina, US; 65 to 100% in surface fresh waters of Lake Wuermsee, Germany; and 35% in Lake Kivu, East Central Africa.

Keywords: Isotopes; geochemistry; microbial methane genesis

Introduction

In nature, microbial methane is produced in water-saturated sediments in two main ways — through acetate fermentation and CO_2 reduction (Koyama et al., 1973; Schoell, 1980; Takai, 1970; Winfrey and Zeikus, 1977) — by the reactions

$$CH_3COOH \rightarrow CH_4 + CO_2, \quad (1)$$

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O. \quad (2)$$

About 70% of natural methane result in process (1), and 30%, in process (2) (Jeris and McCarty, 1965; Koyama, 1963; Schlegel et al., 1976; Smith and Mah, 1966). Both processes can run simultaneously, which often yields mixtures of methane of both types (Burke et al., 1988; Martens et al., 1986). It is difficult to distinguish between acetate fermentation methane and CO_2 reduction methane in geologic objects, because the effect of different factors during the microbial formation of CH_4 has been poorly studied.

Using stable isotopes of hydrogen (D/H) and carbon ($^{13}C/^{12}C$) to distinguish a difference between the above ways of microbial methane formation in geologic objects is based on some empirical factors and is restricted by some poorly substantiated assumptions. For example, Nakai et al. (1974) and Erokhin (1978) revealed an empirical linear relationship between the hydrogen isotope compositions of methane and associated water:

$$\delta D(CH_4) = a\delta D(H_2O) + b, \quad (3)$$

* Corresponding author.
E-mail address: guts@mail.lviv.ua (L.K. Gutsalo)

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where the coefficient a, according to Schoell et al. (1980, 1984), provides the information about what amount of hydrogen in CH_4 molecule was borrowed from water and what amount, from organic matter. The constant b reflects the isotopic fractionation of hydrogen in the system CH_4–H_2O. In the case of CO_2 reduction methane, $a = 1$ (Schoell et al., 1980, 1984; Whiticar, 1999; Whiticar et al., 1986; Woltemate et al., 1984), because water is the only source of hydrogen for CH_4 (Daniels et al., 1980), and $b = -160\pm10‰$ or, according to refined data (Whiticar et al., 1986), $-180\pm10‰$. If methane is produced through CH_3 radical hydrogenation, then $a = 0.25$, because only one hydrogen atom is borrowed from water. When CH_4 forms in the above two ways simultaneously, the a value varies from 1 to 0.25 (Schoell, 1980, 1984; Whiticar, 1999; Whiticar et al., 1986; Woltemate et al., 1984). Note that $a = 0.15$ estimated from the experiment on microbial methane formation through acetate fermentation (Schoell et al., 1988) differs significantly from the theoretically expected values of 0.25, which still has no satisfactory explanation. For this microbial methane formation, the b value depends on several factors (Whiticar, 1986) and is $-318‰$, according to experimental data (Schoell et al., 1988), and $-393‰$, according to calculations (Jenden and Kaplan, 1986).

Using stable carbon isotopes ($^{13}C/^{12}C$) to determine the ways of microbial methane production in nature is based on the statistically recognized difference in the values of the factors of carbon isotope fractionation in the system CO_2–CH_4. In the case of CO_2 reduction, the $\alpha(CO_2–CH_4)$ values usually vary between 1.05 and 1.10 (Whiticar, 1999; Whiticar et al., 1986; Whiticar and Faber, 1985). At the same time, the $\alpha(CO_2–CH_4)$ values for acetate fermentation vary from 1.04 to 1.06 (Whiticar, 1999; Whiticar et al.,1986; Whiticar and Faber, 1985). Whiticar et al. (1986) emphasize that the estimated values of the above factors in the system CO_2–CH_4 are not equilibrium and characterize only carbon isotope ratio. According to these authors, carbon isotope fractionation during microbial methane genesis is generally described by the kinetic isotope effect rather than the isotope exchange equilibrium.

In recent decades, a number of works concerned with various aspects of microbial methane genesis have appeared. However, the criteria for determination of the main ways of this process in the Earth's crust have been poorly studied and substantiated. Most of these criteria are based on empirical relations or statistical basis (Chidthaisong et al., 2002; Fey et al., 2004; Hornibrook, 2000; Sugimoto and Wada, 1995; Valentine et al., 2004; Waldron et al., 1999), which can lead to ambiguous conclusions.

For example, based on experiments in fresh-water medium, Sugimoto and Wada (1995) established the following difference in the isotopic compositions of microbial methane produced through CO_2 reduction and acetate fermentation:

$$\delta D(CH_4 - CO_2) = (0.683 \pm 0.020)\delta D_{water} - (317 \pm 20)$$

and

$$\delta D(CH_4 - Ac) = (0.437 \pm 0.045)\delta D_{water} - (302 \pm 15),$$

respectively.

In the authors' opinion, the a value (0.437) of the relationship δD_{water}—$\delta D(CH_4)$ for acetate fermentation indicates that some hydrogen atoms in CH_3 groups of acetate exchange with those of water on methane genesis.

At the same time, Waldron et al. (1999) made a statistical processing of data for the same fresh-water medium and recognized (independently of the ways of microbial methane genesis) two relationships between $\delta D(CH_4)$ and $\delta D(H_2O)$ for natural and experimental conditions:

$$\delta D(CH_4) = 0.675\delta D(H_2O) - 284‰ \ (p < 0.0001)$$

and

$$\delta D(CH_4) = 0.444\delta D(H_2O) - 321‰ \ (p < 0.0001),$$

respectively.

The authors assumed that methane genesis ways can also influence the isotopic composition of microbial methane, but the basis for this hypothesis must be revised, because the available field and laboratory data do not confirm it.

All the above points to the indefinite and uninformative character of the existing methods (criteria) for estimation of the ways of microbial methane production in nature.

The goal of this work was: (1) to demonstrate the inconsistency of the modern concepts of isotope relationships in the systems CH_4–H_2O and CH_4–CO_2 in natural microbial methane genesis; (2) to develop (on the basis of new concepts) thermodynamic isotope criteria for estimation of the ways of microbial methane genesis in the Earth's crust; (3) to estimate the relative contribution of each of the two ways of the process in geologic objects; and (4) to substantiate (on the thermodynamic basis) the model for the formation of the isotopic composition of microbial methane in nature.

Initial thermodynamic prerequisites

CO_2 reduction methane. Earlier we considered the main factors determining the formation of the isotopic composition of microbial methane through CO_2 reduction (Gutsalo, 2003a,b). Therefore, we report only some conclusions drawn in those works, which are necessary for constructing a model for the formation of the isotopic composition of microbial methane in nature.

Our research (Gutsalo, 2000b) showed that on the natural microbial methane formation through CO_2 reduction, the values of the factor of biologic fractionation of hydrogen isotopes (α^e_D) in the system CH_4–H_2O at a given temperature of the medium fit the known thermodynamic isotope equilibrium (Lyon and Hulston, 1984). In the temperature range of 0 to 100 °C, the equilibrium is approximated by the equation (Gutsalo, 2003b)

$$10^3 \ln \alpha^e_D \ CH_4 - H_2O = -10.37(10^6/T^2) - 76.22, \quad (4)$$

where T(K) is the temperature of the methane genesis medium.

At any temperature, the factor of thermodynamic equilib-

rium fractionation of hydrogen isotopes (α^e_D in (4)) in the system CH_4–H_2O can be presented as

$$\alpha^e_D = (\delta D(CH_4) + 10^3)/(\delta D(H_2O) + 10^3). \quad (5)$$

Transformation of (5) yielded an equation (Gutsalo, 2003b) for calculating the isotopic composition of CO_2 reduction methane:

$$\delta D(CH_4) = \alpha^e_D \delta D(H_2O) + b^e, \quad (6)$$

where $\alpha^e_D = a$ in empirical relation (3),

$$b^e = 10^3(\alpha^e_D - 1). \quad (7)$$

Thus, the assumptions of some researchers (Jenden and Kaplan, 1986; Schoell, 1980, 1984; Sugimoto and Wada, 1995; Whiticar, 1999; Whiticar et al., 1986; Woltemate et al., 1984) that the a value in (3) depicts how much hydrogen in methane molecule was borrowed from water or organic matter are inconsistent (Gutsalo, 2003b). Parameter a is the factor of biologic isotope fractionation, α_i. It reflects the type of distribution of hydrogen isotopes in the microbial system CH_4–H_2O: equilibrium (CO_2 reduction methane) and kinetic (acetate fermentation methane) (Gutsalo, 2003b).

We established (Gutsalo, 2003a) that the methane formation through CO_2 reduction proceeds under a thermodynamic isotope exchange equilibrium established owing to biologic fractionation of isotopes. The values of the factor of biologic fractionation of carbon isotopes (α^e_C) in the system CH_4^{gas}–CO_2^{gas} fit the known thermodynamic isotope equilibrium (Lyon and Hulston, 1984) at a given temperature of the medium. In the range from 0 to 100 °C it is described by the equation (Gutsalo, 2003a)

$$10^3 \ln \alpha^e_C\, CH_4^{gas} - CO_2^{gas} = -4.63(10^6/T^2) - 15.97, \quad (8)$$

where T(K) is the temperature of the methane genesis medium.

In the system CH_4^{gas}–HCO_3^-, the values of the factor of biologic fractionation of carbon isotopes (α^e_{HC}) also reach the above equilibrium at a given temperature of the medium (Gutsalo, 2003a). In the range from 0 to 100 °C, the equilibrium is approximated by the equation (Gutsalo, 2003a)

$$10^3 \ln \alpha^e_{HC} CH_4^{gas} - HCO_3^- = -6.1175(10^6/T^2) - 7.011, \quad (9)$$

where T(K) is the medium temperature.

In (8) and (9), α^e_C and α^e_{HC} can be expressed as

$$\alpha^e_C = (\delta^{13}C(CH_4) + 10^3)/(\delta^{13}C(CO_2) + 10^3) \quad (10)$$

and

$$\alpha^e_{HC} = (\delta^{13}C(CH_4) + 10^3)/(\delta^{13}C(HCO_3^-) + 10^3). \quad (11)$$

Transformation of (10) and (11) yielded equations (Gutsalo, 2003a) similar to (6) for calculation of the carbon isotope composition of microbial methane produced through CO_2 reduction:

$$\delta^{13}C(CH_4) = \alpha^e_C \delta^{13}C(CO_2) + d^e, \quad (12)$$

where

$$d^e = 10^3(\alpha^e_C - 1), \quad (13)$$

$$\delta^{13}C(CH_4) = \alpha^e_{HC} \delta^{13}C(HCO_3^-) + c^e, \quad (14)$$

where

$$c^e = 10^3(\alpha^e_{HC} - 1). \quad (15)$$

Derived Eqs. (4), (6)–(9), and (12)–(15) permit the calculation of the isotopic compositions of both hydrogen and carbon of CO_2 reduction methane for any temperature (0 to 100 °C) of the medium.

Acetate fermentation methane. Sessions and Hayes (2005), who calculated the fractionation of hydrogen isotopes in biogeochemical systems, noted that in recent decades a concept has been accepted, according to which the reagent (δ_r) and the product (δ_p) of component fractionation in biologic systems are related to each other by the equation $\delta_p = \alpha\delta_r + \varepsilon$, where α and ε are the factors of the same fractionation and $\varepsilon = \alpha - 1$. The value of fractionation can be determined from ε, α, or both parameters. But analysis carried out by the researchers showed that these parameters differ significantly from each other, which evidences the incorrectness of the above calculations.

Sessions and Hayes (2005) drew the conclusion that in biologic systems being mixtures of two components (such as CH_4–H_2O and CH_4–CO_2), each of which fractionates throughout the mixing process, there is no unambiguous solution for three unknown variables (two factors of fractionation and uncontrolled mixing of two hydrogen sources).

Below, we consider a new technique for calculating the factors of biologic nonequilibrium fractionation of hydrogen and carbon isotopes in biologic systems during acetate methane genesis, which is based on available experimental data (Fuchs et al., 1979; Games and Hayes, 1976; Schoell, 1984; Schoell et al., 1988) (Table 1).

Our studies (Gutsalo, 2003a,b) showed that the methane production through acetate fermentation in the Earth's crust is characterized mainly by the nonequilibrium biologic fractionation of hydrogen isotopes in the system CH_4–H_2O and carbon isotopes in the system CH_4–H_2O.

The plots in Fig. 1 were constructed using experimental data on the formation of microbial methane through both the acetate fermentation (1) of the Lake Kivu sediments and sewage muds (Schoell, 1984; Schoell et al., 1988; Whiticar, 1986) and CO_2 reduction (in closed system) in the medium free of organic matter (2), where CO_2 was the only source of carbon for methane production (Games et al., 1976, 1978).

Figure 1, *a* shows a linear relationship (r = 0.99) between the hydrogen isotope compositions of water and acetate fermentation methane over a broad range of their δD values. It is expressed by (3) but differs from the thermodynamically equilibrium relationship (6) specific for CO_2 reduction methane. This evidences that in the case of acetate fermentation methane, the hydrogen isotope fractionation in the system CH_4–H_2O is determined mainly by the kinetic factor rather

Table 1
Isotopic fractionation of hydrogen (D/H) and carbon ($^{13}C/^{12}C$) during experimental microbial methane genesis

T, °C	δD(CH_4), ‰	δD(H_2O), ‰	$10^3 \ln \alpha^e_D$	Reference	*T*, °C	Time, h	$\delta^{13}C(CH_4)$, ‰	$\delta^{13}C(CO_2)$, ‰	$10^3 \ln \alpha^e_C$	Reference
Methanosarcina culture with sediments of Lake Kivu					*M. thermoautotrophicum*					
26	–324	–45.0	–346	[Schoell, 1984; Schoell et al., 1988; Whiticar et al., 1986]	65	—	–64.9	–41.4	–24.8	[Games, Hayes, 1976]
26	–315	33.0	–411	The same	65	5.5	–64.5	–39.8	–26.1	The same
26	–303	86.0	–444	The same	20	13	–65.1	–37.0	–29.6	The same
26	–291	181.0	–510	The same	20	24	–65.0	–27.9	–38.9	The same
26	–276	271.0	–563	The same	25	48	–55.3	3.9	–60.8	The same
Sewage muds					25	48	–50.5	9.8	–61.6	The same
60	–348	–54.0	–372	[Schoell, 1984; Whiticar et al., 1988]	60	168	–43.0	–4.7	–39.2	The same
60	–313	9.0	–384	The same	65	—	–37.6	–3.5	–34.8	[Fuchs et al., 1979]
60	–260	179.0	–466	The same	65	—	–40.0	–0.6	–40.2	The same
60	–200	266.0	–459	The same	—	—	—	—	—	—

Note. In the experiments with hydrogen, wide variations in the isotopic composition of water in the medium were provided by addition of D_2O (Schoell, 1980; Schoell et al., 1988).

than the thermodynamic exchange equilibrium. The slope of the lines 1-K and 2-K (α^b_D) to the abscissa axis depends on the temperature of the acetate methane genesis medium (Fig. 1, *a*). It depicts the general factor of the biologic nonequilibrium fractionation of hydrogen isotopes at a given temperature of the medium in accordance with the equation

$$\alpha^b_D = (\delta D_2(CH_4) - \delta D_1(CH_4))/(\delta D_2(H_2O) - \delta D_1(H_2O)), \quad (16)$$

where δD(H_2O) and δD(CH_4) with subscripts 1 and 2 are the corresponding different values of these parameters on the same straight line.

The correctness of (16) is easy to verify. Marking the initial values of δD at the point with index 1 as $\delta D_{init}(H_2O)$ and $\delta D_{init}(CH_4)$ and assuming that the denominator of the fraction in (16) is an increment $\Delta D_{2-1}(H_2O)$ at the segment of the straight line with indices 1 and 2 or 2 and the initial value, we obtain: $\delta D_2(CH_4) = \alpha \Delta D_{2-1}(H_2O) + \delta D_{init}(CH_4)$.

Considering that $a = \alpha^b_D$, (3) can be written

$$\delta D(CH_4) = \alpha^b_D \delta D(H_2O) + b^b, \quad (17)$$

where b^b is the specific factor of the biologic nonequilibrium

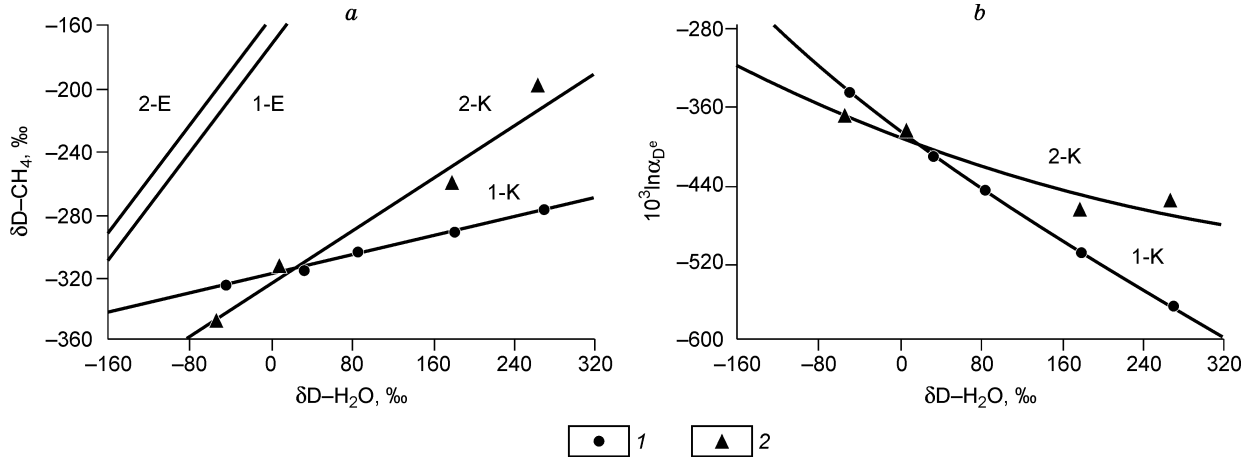

Fig. 1. *a* — Relationship between δD of methane and δD of coexisting water on microbial methane genesis: (1) in equilibrium CO_2 reduction process (calculated from Eqs. (4), (6), and (7)): 1-E — for 26 °C, 2-E — for 60 °C; (2) in experimental nonequilibrium acetate fermentation process (see Table 1): 1-K — for 26 °C ($\delta D(CH_4) = 0.153\delta D(H_2O) - 317.923$, $r = 0.997$, $n = 5$), 2-K — for 60 °C ($\delta D(CH_4) = 0.431\delta D(H_2O) - 323.32$, $r = 0.987$, $n = 4$). *b* — Relationship between the factor of hydrogen isotope fractionation, $10^3 \ln \alpha^e_D$, in the system CH_4–H_2O and δD of coexisting water in experimental nonequilibrium acetate fermentation process (see Table 1).

fractionation of hydrogen isotopes (‰) typical of the particular kind or biocenosis of bacteria.

Note that α_D^b and b^b in (17) are constant values at a given temperature and the $\delta D_{init}(H_2O)$ and, hence, $\delta D_{init}(CH_4)$ are most often variables (except for oceanic water, whose $\delta D_{init}(H_2O)$ value is close to zero). With $\delta D_{init}(H_2O) = 0$, $\delta D_{init}(CH_4)$ is equal to b^b.

Let us determine the temperature dependence of α_D^b for the straight lines 1-K and 2-K (Fig. 1, *a*):

$$10^3 \ln \alpha_D^b = -477.357(10^6/T^2) + 3458.55, \quad (18)$$

where T (K) is the temperature of the methane genesis medium.

Note that in (18), $\alpha_D^b = 1$ at 98.4 °C, i.e., close to 100 °C, which is within the limits of the calculation accuracy. As temperature drops, the isotope fractionation increases, and the value of the factor of nonequilibrium fractionation of hydrogen isotopes (α_D^b) decreases to 0.053 at 0 °C.

Our studies showed that α_D^b in (17) does not depend on the kind of bacteria involved in acetate methane genesis, whereas b^b depends on the kind of bacteria and the medium temperature. As shown below, for the biocenosis of bacteria participating in acetate methane genesis in the anoxic sea water of lagoon basin (North Carolina, US) (Table 2) (Burket et al., 1988), the dependence of b^b on the ambient temperature (7 to 28 °C on the plot and, obviously, even in the wider range 0–100 °C) is expressed as follows:

$$b^b = k(10^6/T^2) + m, \quad (19)$$

where $k = 27.35$, $m = -586.69$, and T (K) is the temperature of the methane genesis medium.

For other kinds of methane-generating bacteria, the same k value as in (19), equal to 27.35, holds, but the value of m is different. For *Methanosarcina barkeri strain* of the Lake Kivu sediments in the East African Rift system (Table 1), $m = -623.53$; for biocenosis of bacteria involved in acetate methane genesis in sewage muds, Germany (Table 1), $m = -569.74$; and for biocenosis of bacteria in Lake Wuermsee, northern Germany, $m = -656.55$ (Table 3).

We have recognized that nonequilibrium isotope fractionation observed for hydrogen in the system CH_4–H_2O during the acetate fermentation production of microbial methane also takes place for carbon in the system CH_4–CO_2 on the experimental methane production through CO_2 reduction (Fig. 2, *a*). As follows from the results of analysis (Fig. 2, *a*), this is because the time of experiment was insufficient for the establishment of a thermodynamic equilibrium usually observed in nature (Gutsalo, 2003a). According to the calculation based on the time dependence of $10^3 \ln \alpha$ (Table 2), a thermodynamic isotope equilibrium can be reached when the experiment lasts for 57 h (instead of 48 h, as in our case) at 20–25 °C (line 1-E in Fig. 2, *b*) and for about 380 h at 65 °C (line 2-E in Fig. 2, *b*).

Taking into account the short duration of the experiment (Table 1) and the state of the system CH_4–CO_2 close to the thermodynamic carbon isotope equilibrium (Fig. 2), the existence of such an equilibrium during natural CO_2 reduction over eons seems obvious and logic.

Figure 2, *a* shows that in the experiment in closed system, the formation of microbial methane through CO_2 reduction first runs at the expense of ^{12}C of CO_2. This process leads (Table 1, Fig. 2, *a*) to the enrichment of residual CO_2 with ^{13}C. As a consequence, the methane produced from it is also enriched with ^{13}C (Fig. 2, *a*). Carbon isotopes in the system CH_4–CO_2 therewith tend to reach a thermodynamic isotope equilibrium (Fig. 2, *a*, *b*).

Comparison of Figs. 1 and 2 shows that the nonequilibrium distribution of carbon isotopes in the system CH_4–CO_2 is similar to that of hydrogen isotopes in the system CH_4–H_2O. Therefore, in studying the behavior of carbon isotopes in the system CH_4–CO_2, we will restrict ourselves to drawing conclusions.

On the investigation of carbon isotope fractionation in the system CH_4–CO_2, one should take into account the phase state of CO_2 and CH_4 and carbonate form of CO_2 in the Earth's crust fluids (Gutsalo, 2003a).

As seen from Fig. 2, *a*, the slope of the lines 1-K and 2-K (α_C^b) to the abscissa axis depends on temperature and depicts the general factor of the biologic nonequilibrium fractionation of carbon isotopes at a given temperature of the methane genesis medium in accordance with the equation

$$\alpha_C^b = (\delta^{13}C_2(CH_4) - \delta^{13}C_1(CH_4))/(\delta^{13}C_2(CO_2) - \delta^{13}C_1(CO_2)), \quad (20)$$

where $\delta^{13}C(CO_2)$ and $\delta^{13}C(CH_4)$ with subscripts 1 and 2 are the different values of these parameters on the same straight line. Change in the temperature of the methane genesis medium from 65 to 25–20 °C leads to a change in the straight-line slope (Fig. 2, *a* and Table 1).

Based on Fig. 2, *a*, $\delta^{13}C(CH_4)$ is written, by analogy with (17), as follows:

$$\delta^{13}C(CH_4) = \alpha_C^b \delta^{13}C(CO_2) + d^b, \quad (21)$$

where d^b is the specific factor of the biologic nonequilibrium fractionation of carbon isotopes (‰) typical of a particular kind or biogenesis of bacteria. With $\delta^{13}C(CO_2) = 0$, $\delta^{13}C(CH_4)$ is equal to d^b.

For the straight lines 1-K and 2-K (Fig. 2, *a*), the temperature dependence of α_C^b is expressed as

$$10^3 \ln \alpha_C^b = -277.954(10^6/T^2) + 1998.677, \quad (22)$$

where T(K) is the temperature of the methane genesis medium.

Note that $\alpha_C^b = 1$ at 99.8 °C, i.e., nearly at the temperature when α_D^b in (18) also equals unity (about 100 °C). The variation limits of the values of the factors of the biologic nonequilibrium fractionation of hydrogen isotopes (α_D^b) in the system CH_4–H_2O and carbon isotopes (α_C^b) in the system

Table 2
Calculated (from Eqs. (24) and (25)) contribution of acetate fermentation methane to the general balance of microbial methane in the surface anoxic marine deposits of lagoon basin, North Carolina, US

Sampling date	T, °C	$\delta D_M(CH_4)$, ‰	$\delta^{13}C_M(CH_4)$, ‰	$\delta^{13}C_M(CO_2)$, ‰	$\delta D_R(CH_4)$, ‰	$\delta D_F(CH_4)$, ‰	$\delta^{13}C_R(CH_4)$, ‰	$\delta^{13}C_F(CH_4)$, ‰	f_D, %	f_C, %
6 June 1983	21.0	—	–64.5	–6.8	—	—	–73.5	–58.5	—	60
19 July 1983	26.5	—	–62.2	–8.6	—	—	–73.4	–57.0	—	68
3 August 1983	27.0	—	–61.7	–8.8	—	—	–73.4	–57.1	—	72
19 August 1983	27.5	–284	–56.8	–9.4	–174	–284	–73.9	–56.9	100	100
14 and 15 September 1983*	28.0	–261	–60.3	–8.3	–174	–285	–72.6	–56.4	78	76
16 October 1983	23.0	–265	–60.0	–7.2	–177	–275	–73.2	–57.8	90	86
20 November 1983	15.0	—	–62.2	–8.0	—	—	–76.7	–61.2	—	94
2 February 1984	7.0	–238	–64.3	–6.0	–188	–238	–77.8	–64.3	100	100
7 April 1984	14.0	–240	–63.8	–5.1	–183	–255	–74.3	–60.9	79	78
6 May 1984	18.0	–231	–63.8	–3.0	–180	–264	–70.9	–58.6	61	58
31 May 1984	23.0	–228	–68.5	–7.0	–177	–275	–80.4**	–57.7	52	52
14 June 1984	23.0	–233	–64.1	–6.2	–177	–275	–72.2	–57.5	57	55
2 July 1984	24.0	–271	–59.4	–10.0	–176	–277	–75.5	–58.3	94	94
18 July 1984	26.0	–263	–60.6	–10.6	–175	–281	–75.4	–57.6	83	83
10 and 11 August 1984*	25.0	–277	–57.3	–7.6	–175	–279	–72.9	–57.2	98	99
30 and 31 August 1984*	26.0	–281	–57.4	–8.9	–175	–281	–73.8	–57.3	100	101
19 and 22 September 1984*	26.0	–276	–58.0	–8.1	–175	–281	–73.0	–57.0	95	94
17 November 1984	16.0	–260	–61.0	—	–181	–260	—	—	100	—

Note. Calculations were performed using the isotope and temperature data of Burke et al. (1988), Crill and Martens (1986), and Martens et al. (1986). Dash means no data.

* Parallel determinations of $\delta D(CH_4)$, $\delta^{13}C(CH_4)$, and $\delta^{13}C(CO_2)$ were not carried out; therefore, we used data with corresponding parameters obtained on the nearest dates (see the references above).

** $\delta^{13}C_R(CH_4)$ were calculated from (14).

CH_4–CO_2 correspond to the temperature range (~0 to 100 °C) in which most microorganisms are usually viable. Temperature higher than 100 °C is fatal to most of them, and temperature lower than –10 °C limits their generation. Drastic temperature changes kill microorganisms. Alternating freezing and thawing are also fatal to bacteria (Beerstecher, 1954). According to Welhan (1988), extrapolation of the experimental data of Coleman et al. (1981) to 100 °C shows that the fractionation of carbon isotopes on the bacterial oxidation of methane virtually vanishes near the boiling point of water (Welhan, 1988).

Thus, the boiling point of water (100 °C) is a biologic isotope datum, an ultimate temperature, above which the life and vital activity of biologic systems and, hence, biologic isotope fractionation cease.

The performed analysis showed that α_C^b in (21) does not depend on the kind of bacteria involved in methane genesis. In contrast to α_C^b, the constant d^b in (21) depends on both the kind of bacteria and the medium temperature. On the experimental CO_2 reduction with the participation of *Methanobacterium thermoautotrophicum* (Table 1, Fig. 2, *a*), the temperature dependence of d^b (in the studied temperature range of 22.5 to 65 °C and, obviously, at higher temperatures, up to 100 °C) is as follows:

$$d^b = n(10^6/T^2) + p, \quad (23)$$

where n = –6.2094, p = 15.989, and T (K) is the temperature of the methane genesis medium.

As shown below (Fig. 3, *b*), for bacterium biocenosis during acetate methane genesis in the anoxic marine deposits of lagoon basin, North Carolina, US (Table 2), n and p in (23) are equal to –5.5489 and 7.673, respectively. Note that the d^b values in (23) calculated from the above parameters differ little from those calculated from the experimental data (cf. lines 1-K and 2-K in Fig. 3, *b*). For bacterium biocenosis during acetate methane genesis in Lake Wuermsee, northern Germany, n = –5.5489 and p = 15.522 (Table 3).

Equations (16)–(23) permit the calculation of the isotopic compositions of hydrogen and carbon of methane produced through acetate fermentation at temperatures of 0 to 100 °C

Table 3
Calculated contribution of acetate fermentation methane to the general balance of microbial methane in the surface fresh-water sediments of Lake Wuermsee, northern Germany

Station	T, °C	Depth, cm	$\delta D_M(CH_4)$	$\delta^{13}C_M(CH_4)$	$\delta D_M(H_2O)$	$\delta^{13}C_M(CO_2)$	$\delta D_R(CH_4)$	$\delta D_F(CH_4)$	$\delta^{13}C_R(CH_4)$	$\delta^{13}C_F(CH_4)$	f_D	f_C
			‰								%	
1	17.5	0–5	–317	–59.5	–21.2	–15.1	–198	–335	–90.3	–54.3	87	86
		5–15	–312	–57.7	–21.2	–14.6	–198	–335	–81.9	–54.2	82	87
2	19.0	0–5	–291	–60.2	–27.9	–3.0	–202	–339	–78.4	–50.3	65	65
		5–15	–296	–60.3	–27.9	–3.2	–202	–339	–78.6	–50.4	69	65
		15–30	–306	–55.7	–27.9	–4.5	–202	–339	–72.0**	–50.8	76	77
		30–50	–294	–57.8	–27.9	–7.9	–202	–339	–75.2**	–51.7	67	74
3	18.8	0–5	–319	–58.0	–27.0	–8.8	–202	–339	–83.9	–52.1	85	81
		5–15	–308	–57.8	–27.0	–9.3	–202	–339	–76.5**	–52.2	77	77
		15–30	–309	–59.9	–27.0	–9.7	–202	–339	–84.7	–52.3	78	78
		30–50	–312	–59.4	–27.0	–7.1	–202	–339	–83.9	–52.1	80	77
4	19.0	0–5	–320	–56.0	–27.9	–11.4	–199	–339	–78.4**	–52.7	86	87
		5–15	–322	–56.3	–27.9	–15.2	–199	–339	–82.0**	–53.8	88	91
		15–30	–322	–57.3	–27.9	–17.1	–199	–339	–83.8**	–54.3	88	90
		30–50	–319	–56.9	–27.9	–9.1	–199	–339	–84.1	–52.1	86	85
5	17.5	0–5	–329	–57.5	–21.8*	–13.8	–198	–335	–89.1	–53.9	96	90
		15–30	–324	–57.4	–21.7	–17.9	–198	–335	–85.0**	–55.1	92	92
6	16.0	0–5	–329	–58.3	–14.4	–21.1	–193	–331	–96.5	–56.4	99	95
		5–15	–331	–58.7	–14.4	–9.8	–193	–331	–86.1	–53.4	101	84
		15–30	–332	–57.0	–14.4	–13.5	–193	–331	–89.5	–54.4	100	93
		30–50	–334	–55.3	–14.4	–14.4	–193	–331	–90.3	–54.7	102	98
7	17.5	0–5	–314	–59.0	–21.2	–15.6	–198	–335	–82.9**	–54.4	85	84
		5–15	–316	–59.5	–21.2	–16.1	–198	–335	–91.2	–54.6	86	87
		15–30	–318	–58.9	–21.2	–13.8	–198	–335	–89.1	–53.9	87	86
		30–50	–320	–59.0	–21.2	–10.8	–198	–335	–86.3	–53.1	89	82
8	17.5	0–5	–326	–58.4	–21.2	–10.8	–198	–335	–86.3	–53.1	93	84
		5–15	–327	–58.6	–21.2	–11.2	–198	–335	–86.7	–53.2	94	84
		15–30	–327	–58.9	–21.2	–10.8	–198	–335	–86.3	–53.1	94	83
		30–50	–322	–60.0	–21.2	–11.6	–198	–335	–87.1	–53.3	90	80
9	15.5	0–5	–313	–59.2	–12.3	–14.1	–192	–330	–90.3	–54.8	88	88
		5–15	–315	–56.7	–12.3	–12.7	–192	–330	–80.9**	–54.4	89	91
10	17.5	0–5	–323	–58.2	–21.2	–11.5	–198	–335	–87.0	–54.2	91	88
		5–15	–326	–58.7	–21.2	–12.5	–198	–335	–87.9	–53.6	93	85
		15–30	–335	–58.2	–21.2	–10.9	–198	–335	–86.4	–53.2	100	85
		30–50	–334	–58.9	–21.2	–13.0	–198	–335	–88.4	–53.7	99	85
11	19.0	5–15	–325	–55.9	–27.9	—	–202	–339	—	—	90	—
12	18.0	0–5	–327	–58.3	–20.0*	–10.3	–196	–336	–85.6	–52.8	94	83
		5–15	–330	–58.7	–25.0*	–11.5	–201	–337	–86.8	–53.1	96	83
13	18.0	0–5	–321	–53.9	–23.4	–5.1	–199	–337	–72.9**	–51.4	88	88
		5–15	–323	–54.9	–23.4	–4.7	–199	–337	–80.5	–51.1	90	87
		15–30	–323	–54.0	–23.4	–3.6	–199	–337	–79.4	–50.9	90	89
		30–50	–325	–54.8	–23.4	–3.7	–199	–337	–79.5	–51.0	91	87
14	18.5	0–5	–333	–53.4	–25.7	–7.6	–201	–338	–82.9	–51.8	96	95
		5–15	–334	–53.9	–25.7	–7.9	–201	–338	–83.2	–51.9	97	94
		15–30	–338	–52.1	–25.7	–8.5	–201	–338	–83.7	–52.1	100	100
		30–50	–332	–52.5	–25.7	–9.8	–201	–338	–84.9	–52.5	96	100
15	18.5	0–5	–328	–60.4	–24.6*	–14.0	–200	–338	–88.8	–53.6	93	81
		5–15	–331	–60.5	–27.3*	–12.4	–202	–338	–87.3	–53.3	95	79
		15–30	–334	–57.8	–25.7	–13.5	–201	–338	–88.4	–53.5	97	88
		30–50	–333	–58.0	–25.7	–15.4	–201	–338	–90.1	–54.0	96	89
16	19.0	200	–324	–64.1	–28.1*	–15.3	–202	–339	–89.8	–53.8	89	71

Note. Isotope and temperature data were taken from (Whiticar et al., 1986; Woltemate et al., 1984).

* Measured $\delta D_M(H_2O)$ values. For the other samples, $\delta D_M(H_2O)$ values were calculated from the established temperature dependence $\delta D_M(H_2O) = 54.87(10^6/T^2) - 670.73$, $r = 0.814$, $n = 7$.

** For these samples, $\delta^{13}C_R$ were calculated from (8), and for the others, from (9).

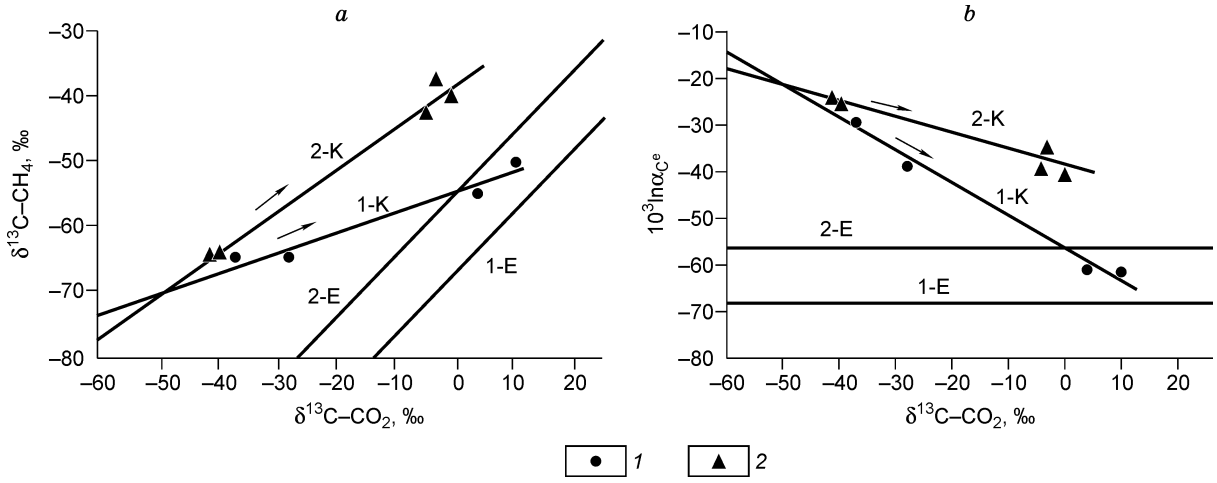

Fig. 2. *a* — Relationship between $\delta^{13}C$ of methane and $\delta^{13}C$ of coexisting CO_2 on microbial methane genesis: (1) in equilibrium CO_2 reduction process (calculated from Eqs. (8), (12), and (13)): 1-E — for 22.5 °C, 2-E — for 65°C; (2) in experimental nonequilibrium CO_2 reduction process in closed system (see Table 1): 1-K — for 22.5 °C ($\delta^{13}C(CH_4) = 0.307\delta^{13}C(CO_2) - 55.046$, $r = 0.974$, $n = 4$), 2-K — for 65 °C ($(\delta^{13}C(CH_4) = 0.649\delta^{13}C(CO_2) - 38.312$, $r = 0.991$, $n = 5$). *b* — Relationship between the factor of carbon isotope fractionation, $10^3 \ln \alpha^e_C$, in the system CH_4–CO_2 and $\delta^{13}C$ of coexisting CO_2 during microbial methane genesis: (1) in equilibrium CO_2 reduction process (calculated from (10)); (2) in experimental nonequilibrium CO_2 reduction process (Table 1). Arrows show the process evolution in time.

for a particular kind or biocenosis of bacteria involved in the process.

For the nonequilibrium fractionation of carbon isotopes in the system CH_4–CO_2 during both the experimental CO_2 reduction and natural acetate fermentation microbial methane genesis (lines 1-K and 2-K in Fig. 3, *b*), the close calculated values of d^b in (23) suggest a similar biologic distribution of isotopes in nonequilibrium processes.

We have established that during acetate methane genesis at a given temperature, the lower is the $\delta D(H_2O)$ value of initial water, the closer to the thermodynamic isotope equilibrium is the hydrogen isotope distribution in the system CH_4–H_2O (Fig. 1, *a*). And vice versa, the higher is $\delta^{13}C$ of initial CO_2, the closer to the thermodynamic isotope equilibrium is the carbon isotope distribution in the system CH_4–CO_2 (Fig. 2, *a*).

We emphasize that the equations for calculating the factors of nonequilibrium biologic ((16) and (20)) and equilibrium thermodynamic ((5), (10), and (11)) fractionation of hydrogen isotopes in the system CH_4–H_2O and carbon isotopes in the system CH_4–CO_2 are nonidentical and differ considerably. Therefore, the use of (5), (10), and (11) (Chidthaisong et al., 2002; Fey et al., 2004; Hornibrook et al., 2000; Jenden and Kaplan, 1986; LaZerte, 1981; Sugimoto and Wada, 1995; Whiticar, 1999; Whiticar et al., 1986; Waldron, 1999; Woltemate et al., 1984) for calculating the factors of nonequilibrium fractionation of isotopes of hydrogen, carbon, and, probably, other elements in biologic systems is wrong. It leads to erroneous conclusions about the dependence of the values of isotope fractionation factors ($10^3 \ln \alpha$) on the initial isotopic composition of H_2O or CO_2 at the same temperature, as well seen from Figs. 1, *b* and 2, *b*, which in the case of hydrogen cannot be accounted for by the different degrees of substrate exhaustion (see Note to Table 1).

Application of the model to natural objects

The above model for the biologic nonequilibrium distribution of hydrogen and carbon isotopes in the systems CH_4–H_2O and CH_4–CO_2 on acetate methane genesis is based mainly on experimental data. Below, we present results of the application of this model to some natural objects.

We invoked, first of all, data on the anoxic marine deposits of lagoon basin (North Carolina, US), which were obtained by parallel determinations of the isotopic compositions of hydrogen in the system CH_4–H_2O and carbon in the system CH_4–CO_2 (Burke et al., 1988; Crill and Martens, 1986; Martens et al., 1986).

As seen from Fig. 3, *a*, the field of factual data (Table 2) (Burke et al., 1988) is restricted below by the straight line 1-K, which depicts an intimate relationship between the hydrogen isotope composition of methane and the temperature of acetate dissimilation in the temperature range from 7 to 28 °C. The chaotic field of points between the lines 1-K and 1-E reflects the mixing of methane produced through acetate fermentation with that resulted from CO_2 reduction in different proportions.

The hydrogen isotope compositions of CO_2 reduction methane (line 1-E) and acetate fermentation methane (line 1-K) show opposite dependences on the temperature of the methane genesis medium (Fig. 3, *a*).

The same factual data (Table 2) show that the carbon isotope composition of acetate fermentation methane also depends on the temperature of the medium of its genesis (Fig. 3, *b*). This is due to the fact that on the nonequilibrium distribution of isotope of one element, the same regularity must also be observed for isotopes of other elements involved in the process. The field of points is restricted above by the line 1-K depicting the relationship of the carbon isotope composition of methane with the temperature of the medium

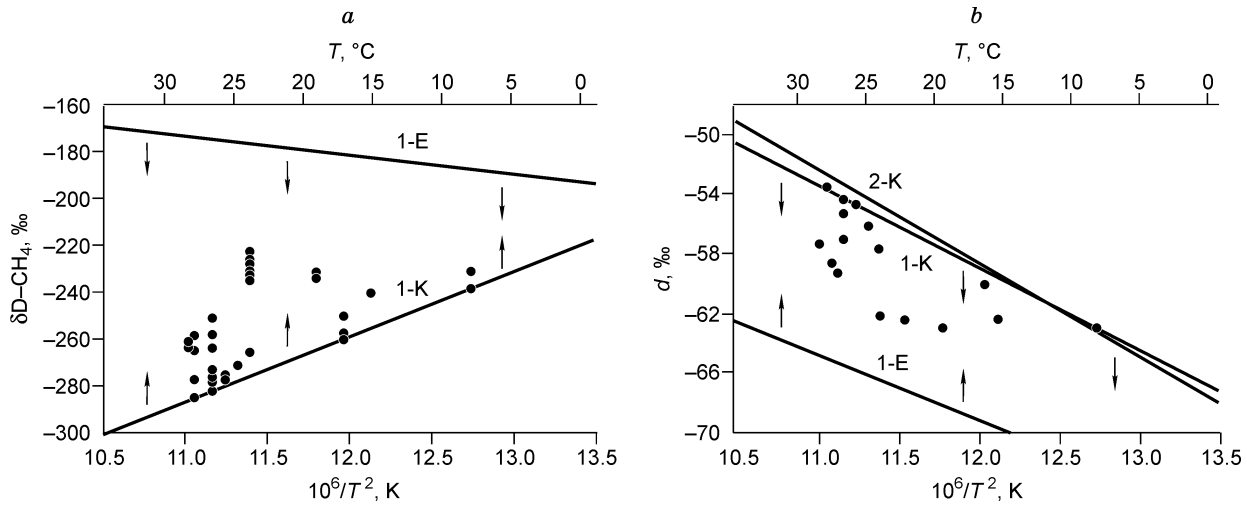

Fig. 3. *a* — Relationship between δD of methane and the temperature of the medium of microbial methane genesis in the anoxic marine deposits of lagoon basin (North Carolina, US): 1-E — in equilibrium CO_2 reduction process (calculated from Eqs. (4)– (7)) given that $\delta D(H_2O)$ in (6) equals zero; 1-K — in nonequilibrium acetate fermentation process in the same anoxic marine deposits (see Table 2): $\delta D(CH_4) = 27.35(10^6/T^2) - 586.69$. *b* — Relationship between d (d^e in Eq. (12) and d^b in Eq. (21)) and the temperature of the microbial methane genesis medium in the anoxic marine deposits of lagoon basin: 1-E — in equilibrium CO_2 reduction process (calculated from Eqs. (8), (10), (12), and (13)) given that $\delta^{13}C(CO_2)$ in (12) equals zero; 1-K — in nonequilibrium acetate fermentation process in the same anoxic marine deposits: $d^b = -5.5489(10^6/T^2) + 7.673$; 2-K — in experimental nonequilibrium CO_2 reduction process in closed system (see Table 1) (calculated from Eq. (23)).

of acetate methane genesis, which is expressed via the parameter d. In contrast to $\delta D(H_2O)$, the parameter $\delta^{13}C(CO_2)$ is variable. Therefore, in Fig. 3, *b* we marked only the $\delta^{13}C(CH_4)$ values equal to the constant d^b calculated from (21), using the factual $\delta^{13}C(CO_2)$ values (Table 2) and the α_C^b value obtained from (22). For comparison, we also drew the straight line 2-K depicting d^b variations, which was calculated from the experimental data (Table 1) obtained from (23). Between the lines 1-K and 1-E (Fig. 3, *b*), mixtures of methane of two types of genesis in different proportions (Table 2) are localized.

To verify the correctness of the proposed model for the formation of the isotopic composition of microbial methane and the established thermodynamic relationships, we can use the general isotope balance equation (Jenden and Kaplan, 1986)

$$\delta A_M = f\delta A_F + (1 - f)\delta A_R, \quad (24)$$

where δA_M is the measured isotopic composition of hydrogen (δD_M) or carbon ($\delta^{13}C_M$) of methane, δA_F is the isotopic composition of hydrogen (δD_F) or carbon ($\delta^{13}C_F$) of acetate fermentation methane, δA_R is the isotopic composition of hydrogen (δD_R) or carbon ($\delta^{13}C_R$) of CO_2 reduction methane, f is the fraction of acetate dissimilation methane calculated from the balance of hydrogen (f_D) or carbon (f_C) isotopes of methane by the equation

$$f = (\delta A_M - \delta A_R)/(\delta A_F - \delta A_R). \quad (25)$$

Table 2 presents the measured isotopic compositions of hydrogen (δD_M) and carbon ($\delta^{13}C_M$) of methane in the anoxic marine deposits of the lagoon basin at a given temperature. The δD_R values for hydrogen isotopes and the $\delta^{13}C_R$ values for carbon isotopes of methane were calculated from (4), (6)–(9), and (12)–(15). The isotopic compositions of hydrogen (δD_F) and carbon ($\delta^{13}C_F$) of acetate fermentation methane were calculated from (17)–(19) and (21)–(23). Since $\delta D(H_2O)$ for pore waters of the studied lagoon basin is close to 0 (Burke et al., 1988), Eqs. (6) and (17) are simplified; thus, $\delta DR(CH4) = b^e$ (Fig. 3, *a*) and $\delta D_F(CH_4) = b^b$. The calculation results for all components of the isotope balance of microbial methane are given in Table 2.

The calculation results show nearly the same fractions of acetate fermentation methane calculated from the isotopic compositions of both hydrogen (f_D) and carbon (f_C) in the general balance of methane of the lagoon basin (Table 2). In different years and seasons they vary over a broad range of values, from 52–60% in May–June to 70–100% in July–August. But from middle September, 1983, to early February, 1984, the fraction of acetate fermentation methane increased from 77 to 100% (Table 2). At the same time, radioactive ^{14}C tracer data show that its fraction in the general balance of microbial methane of the lagoon basin in July–August, 1983 was as low as 20 to 29% (Martens et al., 1986), which obviously points to the incorrectness of the calculations.

It was established that variations in the isotopic compositions of hydrogen and carbon of microbial methane in different years are mainly due to changes in the temperature of the methane genesis medium and mixing (in different proportions) of methane produced through acetate fermentation with that resulted from CO_2 reduction.

The same fractions of acetate fermentation methane calculated from the isotopic compositions of hydrogen (f_D) and carbon (f_C) in the general balance of methane (Table 2) unambiguously prove the correctness of the proposed model

for the natural formation of the isotopic composition of microbial methane.

We carried out a similar calculation of the hydrogen and carbon isotope balance for microbial methane of the surface fresh waters of Lake Wuermsee (northern Germany), using data of Whiticar et al. (1986) and Woltemate et al. (1984). The results (Table 3) show that this methane is mainly a mixture (in different proportions) of two its kinds of different geneses, with acetate fermentation methane making up 65 to 100%, which agrees with earlier conclusions (Woltemate et al., 1984). In the studied set of samples (Table 3), the average discordance between the mixture compositions calculated from the hydrogen and carbon isotope balance of methane is no more than 5%. The discordance value falling beyond the calculation error range might be accounted for by using mainly the calculated $\delta D(H_2O)$ parameters (see Note to Table 3) and by the applied gas sampling procedure (Woltemate et al., 1984), which can influence the carbonate system. Analysis showed (Table 3) that CO_2 released from water-saturated sediment samples is present in aqueous solution mainly as HCO_3^-.

Of special interest is study of the isotopic balance of microbial methane of Lake Kivu, East Central Africa, because the results of model experiments (Schoell et al., 1988) and calculations (Jenden and Kaplan, 1986) contradict the theoretical prerequisites of some researchers (Jenden and Kaplan, 1986; Schoell, 1980, 1984; Sugimoto and Wada, 1995; Whiticar, 1999). The measured $\delta D_M(CH_4)$ value of the Kivu methane is –218‰, and the lake water is characterized by $\delta D(H_2O)$ = +11‰ (Schoell et al., 1988). For the Kivu water temperature of +26 °C (Whiticar, 1986), δD_R of CO_2 reduction methane, estimated from (4), (6), and (7), is –166‰, and δD_F of acetate fermentation methane, calculated from (17)–(19), is –316‰. In accordance with hydrogen isotope balance equation (25), the fraction of the Kivu methane produced through acetate dissimilation (f_D) is 35%, which is close to the earlier statistically estimated values (33–41%) (Schoell et al., 1988).

We could not calculate the carbon isotope balance of the Kivu microbial methane on the thermodynamic basis because of the lack of our experimental and literature data on the carbon isotope composition of CO_2 in the lake, which varies greatly with space and time (Deuser et al., 1973; Schoell et al., 1988).

On the basis of all the above data, we have concluded that our model for the formation of the isotopic composition of microbial methane in nature is correct and the established isotope relationships are valid.

Conclusions

On the basis of studies of the distribution of hydrogen and carbon isotopes (D/H and $^{13}C/^{12}C$) in the microbial systems CH_4–H_2O and CH_4–CO_2, we have developed (on thermodynamic basis) new isotope criteria for estimating the ways of formation of microbial methane in the Earth's crust. For methane produced through CO_2 reduction, the biologic distribution of hydrogen and carbon isotopes in these systems corresponds to the thermodynamic isotope exchange equilibrium at a given temperature of the methanogenic medium. For acetate fermentation methane, the above systems show mainly a nonequilibrium distribution of hydrogen and carbon isotopes. Based on thermodynamic data, we proposed a model for the formation of the isotopic composition of microbial methane in nature. Variations in the hydrogen and carbon isotope compositions of microbial methane in various geologic objects in marine and fresh-water deposits are due mainly to the variations in the temperature of the methanogenic medium and the mixing (in different proportions) of methane of different geneses.

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