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ANAEROBIC OXIDATION OF METHANE BY NITRATE: THE KINETIC ISOTOPE EFFECT

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The ratio of stable carbon isotopes ($^{13}C/^{12}C$) in different environments serves as a significant limitation in estimating the global balance of methane [Hornibrook et al., 2000]. In this case, the value of $^{13}C/^{12}C$ largely depends on the kinetic isotope effect associated with the metabolism of microorganisms that produce and consume CH_4. The article suggests a dynamic model of the processes of methane formation and its anaerobic oxidation with nitrate by methanotrophic denitrifying microorganisms (DAOM), which allowed estimating the fractionation factor of stable carbon isotopes. In the experiment with peat from the minerotrophic bog [Smemo, Yavitt, 2007], the dynamics of the amount of methane and $\delta^{13}CH_4$ was measured. The dynamic model showed that the introduction of nitrate leads to a slow decrease in the partial pressure of methane. Since methane in the DAOM process is a substrate, methane is enriched with heavier carbon ^{13}C in the system under study. This leads to an increase in the value $\delta^{13}C$-CH_4.The carbon isotope fractionation factor during methane oxidation with nitrate was equal to 1.018 and comparable with the fraction of carbon isotope fractionation in the process of acetoclastic methanogenesis (1.01). Model calculations have shown that during incubation the apparent fractionation factor of carbon isotopes with the simultaneous formation of methane and DAOM slowly decreases. The ratio of $^{13}C/^{12}C$ isotopes in dissolved and gaseous methane practically does not differ. The model showed that an increase in the initial concentration of nitrate increases the rate of DAOM, which leads to a decrease in the concentration of dissolved methane. In this case, the value of $^{13}C/^{12}C$ increases. In field studies, Shi et al. (2017) showed that the presence of DAOM in peat bogs in which fertilizers penetrate can be controlled by the amount of nitrate used and the depth of penetration into the anoxic layer. Two MATLAB files describing DAOM are attached to the article[1].

Key words: methane formation; anaerobic methane oxidation; microorganisms; nitrate; kinetic isotope effect.

Отношение стабильных изотопов углерода ($^{13}C/^{12}C$) служит важным фактором при оценке глобального баланса метана [Hornibrook et al., 2000]. При этом величина $^{13}C/^{12}C$ в значительной степени зависит от кинетического изотопного эффекта, связанного с метаболизмом микроорганизмов, которые производят и потребляют CH_4. В статье предлагается динамическая модель процессов образования метана и его анаэробного окислени я нитратом метанотрофными денитрифицирующими микроорганизмами (DAOM), что позволило оценить коэффициент фракционирования стабильных изотопов углерода. В эксперименте с торфом из минеротрофного болота [Smemo, Yavitt, 2007], измерялась динамика количества метана и величины $\delta^{13}CH_4$. Предлагаемая нами модель показала, что введение нитрата приводит к медленному снижению парциального давления метана. Поскольку метан в процессе DAOM является субстратом, в исследуемой системе происходит обогащение метана более тяжелым углеродом ^{13}C. Это приводит к возрастанию величины $\delta^{13}C$-CH_4. Коэффициент фракционирования изотопов углерода в процессе окисления метана нитратом оказался равным 1.018 и сопоставимым с коэффициентом фракционирования изотопов углерода в процессе ацетокластического метаногенеза (1.01). Модельные расчеты показали, что кажущийся коэффициент фракционирования изотопов углерода при одновременном образовании метана и DAOM в ходе инкубации медленно снижается. Отношение изотопов $^{13}C/^{12}C$ в растворенном и газообразном метане практически не отличаются.

Ключевые слова: образование метана; анаэробное окисление метана; микроорганизмы; нитрат; кинетический изотопный эффект.

INTRODUCTION

Methane is an important greenhouse gas, and understanding of the mechanisms that influence methane formation and oxidation is a necessary element for the transition from local to global level of estimates of methane emissions into the atmosphere [Bridgeham et al., 2013]. Cellulose is the major organic substance in most natural ecosystems [Lynd et al., 2002] that transforms to methane and carbon dioxide. The ratio of stable carbon isotopes ($^{13}C/^{12}C$) of methane is an important factor in assessing the global balance of methane [Hornibrook et al., 2000]. The value of $^{13}C/^{12}C$

[1] Supplementary materials are available here https://edgccjournal.org/EDGCC/rt/suppFileMetadata/10534/0/4975
Дополнительные материалы к статье размещены https://edgccjournal.org/EDGCC/rt/suppFileMetadata/10534/0/4975

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largely depends on the kinetic isotope effect associated with the metabolism of microorganisms that produce and consume CH_4.

The kinetic isotope effect consists in changing the rate of a chemical reaction when an atom is replaced by an isotope in a molecule of a reacting substance [Galimov, 1973, p. 4]. When making corrections for the kinetic isotope effect, the weighted sum of the $^{13}C/^{12}C$ ratios in methane coming from various water bodies should be equal to the ratio for the atmospheric methane. However, in some anaerobic environments, a wide range of $\delta^{13}C\text{-}CH_4$ values often occurs, making it difficult to estimate the average value for each water body. The simultaneous proceeding of processes such as the formation and oxidation of methane significantly affects this value. In addition, it is necessary to take into account the kinetics of methane oxidation in air, which also affects the $^{13}C/^{12}C$ ratio of atmospheric methane. Understanding the nature and causes of $\delta^{13}C\text{-}CH_4$ changes provides the basis for the use of balance estimates of changes in CH_4 in the atmosphere [Hornibrook et al., 2000].

Since the 'heavy' isotope is less abundant compared to the 'light' one, the isotope ratio is usually expressed through the established international standards in ppm (‰) [Craig, 1957]:

$$\delta[‰] = 10^3 \left(\frac{R}{R_{std}} - 1 \right), \quad (1)$$

where R and R_{std} isotope ratios (^{13}C / ^{12}C) in the sample and standard respectively.

The Rayleigh equation [Rayleigh, 1898] originally proposed to describe the fractionation during the diffusion of a gas mixture, is also used to calculate the fractionation of stable isotopes:

$$R_t / R_0 = (S_t / S_0)^{(1/\alpha - 1)}, \quad (2)$$

where R_0 and S_t, S_0 and S_t are the ratio of isotopes in the substrate and the concentration of substrates at the beginning of the reaction and at the time t respectively; α is the coefficient of isotope fractionation during the transformation of a substrate into a product in a closed and perfectly mixed system. Equation (2) is traditionally derived, assuming the validity of the reaction of the 1st order for the concentration of substrate with light and heavy isotopes. In this case, the isotope fractionation coefficient is defined as

$$\alpha = k_{light} / k_{heavy}, \quad (3)$$

which represents the ratio of the rate constants of the reaction of the 1st order for a substrate with a light and a heavy isotope. The reaction for the light isotope substrate is somewhat faster than the reaction with the heavy isotope. During the reaction, the value can be considered constant with good accuracy, which led to the generally accepted opinion about the prevalence of 1st order reactions in isotopic transformations. However, the Rayleigh equation, in fact, does not describe the dynamics of the process [Vavilin, Rytov, 2015].

Assuming that the concentration of the ^{12}C light isotope substrate is much greater than the concentration of the substrate with the heavier ^{13}C isotope, the fractionation equations for the substrate, products, and microbial biomass can be written as

$$\frac{d\,{}^{13C}S}{dt} \approx -\frac{1}{\alpha}\frac{{}^{13C}S}{S}\rho(S,B),$$
$$\frac{d\,{}^{13C}P}{dt} \approx +\frac{1}{\alpha}\frac{{}^{13C}S}{S}(1-Y)\rho(S,B),$$
$$\frac{d\,{}^{13C}B}{dt} \approx +\frac{1}{\alpha}\frac{{}^{13C}S}{S}Y\rho(S,B), \quad (4)$$

where S, P, B are the total concentrations of substrate, product corrected for biomass growth, and the biomass itself, containing both ^{12}C and ^{13}C; ρ (S, B) — the rate of substrate consumption by biomass, ^{13C}S, ^{13C}P, ^{13C}B — the concentration of substrate, product and biomass with ^{13}C; α – the fractionation factor of substrates containing isotopes ^{12}C и ^{13}C; Y – yield coefficient associated with the growth of biomass and reflecting the share of the transformation of the substrate into biomass.

Thus, the dynamics of substrate, product and biomass with the heavier isotope can be expressed through the dynamics of a common substrate without considering its isotopic composition [Vavilin et al., 2017; Vavilin et al., 2018 (a, b)], and the dynamics of fractionation of stable isotopes is a consequence of the dynamics of chemical and biological processes. The redistribution of stable isotopes makes it possible to specify the metabolic pathway of the substrate utilization and determine the corresponding kinetic parameters and fractionation factors.

In the process of methane formation from cellulose material $(C_6H_{10}O_5)_n$ several types of microorganisms take part, carrying out the depolymerization of cellulose, enzymatic acidogenesis, acetogenesis and methanogenesis. The main product of the enzymatic decomposition of cellulose represents monosaccharides that are further transformed into hydrogen (H_2), carbon dioxide (CO_2) and volatile fatty acids (VFA) such as acetate (CH_3COOH):

$$C_6H_{10}O_5 + 3H_2O \rightarrow$$
$$\rightarrow 2CH_3COOH + 2CO_2 + 4H_2. \quad (5)$$

The main substrates for methanogenic microorganisms are acetate and hydrogen + carbon dioxide, respectively [Zinder, 1993]:

$$CH_3COOH \rightarrow CH_4 + CO_2, \quad (6)$$
$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O. \quad (7)$$

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From equations (5)–(7), it follows that the contribution of acetoclastic methanogenesis is 2/3 of the total methane production [Conrad, 2005].

In the works [Vavilin et al, 2017; Vavilin et al., 2018 (a, b)], it has been showed that the contribution of acetoclastic methanogenesis changes significantly with a significant role of such processes as syntropic oxidation of acetate and homoacetogenesis. Since the fractionation of carbon isotopes as a result of acetoclastic and hydrogenotrophic methanogenesis is very different, the following expression for the magnitude of the apparent fractionation coefficient is often used to determine the dominant pathway for methane formation [Whiticar, 1999]:

$$\alpha_c^{app} = \frac{\delta^{13}C_{CO_2} + 1000}{\delta^{13}C_{CH_4} + 1000}. \quad (8)$$

In [Vavilin et al., 2017; Vavilin et al., 2018 a], a mathematical description of the dynamics of cellulose conversion to methane and carbon dioxide in bottom sediments of tropical lakes and peat from boreal bogs, including the redistribution of a stable isotope, has been proposed.

The formation and oxidation of methane by prokaryotes is considered in a review [Kallistova et al., 2017]. Anaerobic oxidation of methane is an important process controlling the release of methane into the atmosphere in marine and freshwater ecosystems. This process takes place in connection to processes such as sulfate reduction, denitrification, and reduction of ferric iron [Bridgeham et al., 2013]. The intracellular pathway of methane oxidation and nitrite reduction by methanotrophic denitrifying culture of Candidatus *Methylomirabilis oxyfera* has been discovered by Ettwig et al. [Ettwig et al., 2010].

Rasigraf et al. [2012] investigated this reaction (DAOM) by measuring isotopes ^{13}C and ^{2}H. Vavilin and Rytov [2016] used their data when modeling the process. For mathematical description, the following stoichiometric reaction has been used:

$$CH_4 + \frac{8-23Y}{3}NO_2^- = Y\,C_5H_7NO_2 + \frac{4-13Y}{3}N_2 + (1-5Y)CO_2 + \frac{8-23Y}{3}OH^- + \frac{2+Y}{3}H_2O, \quad (9)$$

where Y is the biomass yield coefficient, reflecting the partial transfer of substrates to biomass. The Monod function with two substrates limiting the total rate has been used to describe the growth of biomass, the consumption of substrates (methane and nitrite) and the formation of products (nitrogen and carbon dioxide) in accordance with reaction (9). The process of mass exchange of methane and carbon dioxide in liquid and gas phases has been taken into account as well as inhibition by an increased concentration of nitrite.

PROBLEM SETTING

The possibility and potential mechanism of DAOM in some northern ombrotrophic and minerotrophic fens is discussed in Smemo and Yavitt [2007]. In a later work [Smemo, Yavitt, 2011] it has been reported that, without taking into account the processes of anaerobic oxidation of methane, the emission of methane into the atmosphere may be easily overestimated.

The main goal of our work was to develop a dynamic model of DAOM in the anaerobic system, in that methane formation and oxidation processes take place at the same time, based on experimental data from Smemo and Yavitta [2007]. Since microbiological processes take place in water, and the value of $^{13}C/^{12}C$ is usually measured in gas (too small values of dissolved methane), the mass exchange between the gas and liquid phases was taken into account in the proposed mathematical model. According to the model, the isotope ratio of $^{13}C/^{12}C$ in dissolved and gaseous methane is practically the same.

As a result, the model allowed to evaluate the effect of various parameters on the DAOM process and to determine the fractionation factor of stable carbon isotopes.

MATERIALS AND METHODS

Experimental data

A detailed description of the experiments has been given earlier [Smemo, Yavitt, 2007]. The fen of Michigan Hollow (New York, USA), fed by groundwater (minerotrophic fen), was studied. The predominant plant species were *Carex laucustris* and *Typha latifollia*. During batch experiments (six replications) at 25°C with a 250 ml vessel and a volume of liquid containing peat from a depth of 5–15 cm, approximately equal to 150 ml, the dynamics of methane and the $\delta^{13}CH_4$ value were measured. To initiate the DAOM process, initially a certain amount of methane was introduced into the gas phase, and nitrate with an initial concentration of 10 mM was added to the liquid phase. The control was an experiment without addition of nitrate.

Scheme of methane formation and oxidation with nitrate

The general scheme of the formation of methane and its oxidation with nitrate is shown in Figure 1. The mathematical model used below does not

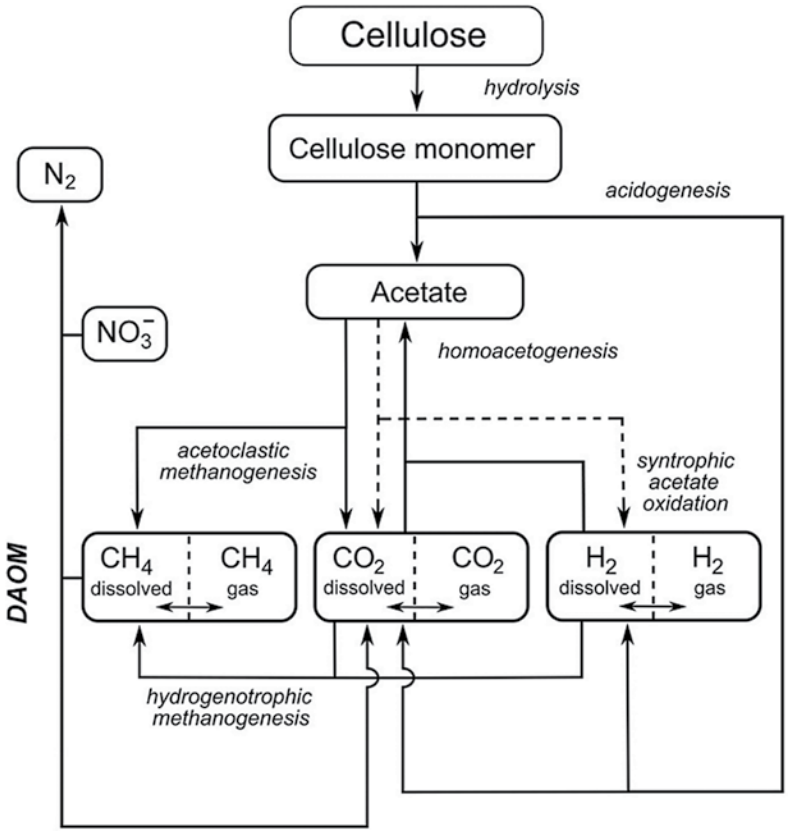

Fig. 1. Scheme of the parallel formation of methane from cellulose and anaerobic oxidation of methane by nitrate (DAOM). In the mathematical model, the processes of synthrophic acetate oxidation and homoacetogenesis are not considered

Рис. 1. Схема совместного образования метана из целлюлозы и анаэробного окисления метана нитратом (DAOM). Для исследуемой системы в математической модели процессы синтрофного окисления ацетата и гомоацетогенеза не рассматриваются

consider the processes of syntrophic acetate oxidation and homoacetogenesis, presented in Fig. 1. For simplicity, in the model the same microbial biomass formula $C_5H_7NO_2$ is used [Rittmann, McCarty, 2001] for various microorganisms.

Cellulose methane production model

The dynamic model previously published was used [Vavilin et al., 2017; Vavilin et al., 2018 (a, b)]. To describe the process of methane formation from acetate carried out by acetoclastic methanogens, the following stoichiometric equation was considered:

$$CH_3COOH + Y\,NH_4^+ = Y\,C_5H_7NO_2 + (1-2.5Y)\,CO_2 + (1-2.5Y)\,CH_4 + 3Y\,H_2O + Y\,H^+, \quad (10)$$

where the stoichiometric coefficient (1–2.5Y) is obtained from the equality of the number of atoms of the chemical elements C, H and O in the right and left sides of the equation (10). Two groups of acetoclastic methanogens *Methanosarcina* and *Methanosaeta* were used in the model to describe acetoclastic methanogenesis.

To describe the formation of methane from carbon dioxide and hydrogen carried out by hydrogenotrophic methanogens, the following stoichiometric equation was considered:

$$CO_2 + (4-10Y)\,H_2 + Y\,NH_4^+ = Y\,C_5H_7NO_2 + (1-5Y)\,CH_4 + (2-2Y)\,H_2O + Y\,H^+, \quad (11)$$

where the stoichiometric coefficients (4–10Y), (1–5Y) and (2–2Y) are obtained from the equality of the number of atoms of the chemical elements C, H and O in the right and left sides of equation (11).

Admitting that the ammonium concentration does not limit the rate of acetoclastic methanogenesis, the corresponding Monod functions can be written as:

$$\rho_{Sar} = \rho_{m\,Sar}\, B_{Sar}\, \frac{Ac}{K_{Sar} + Ac}, \quad (12)$$

$$\rho_{Sae} = \rho_{m\,Sae}\, B_{Sae}\, \frac{Ac}{K_{Sae} + Ac}, \quad (13)$$

where Ac are the acetate concentrations;
B_{Sar}, B_{Sae} — are the concentrations of acetoclastic methanogens *Methanosarcina* and *Methanosaeta* (they are known to be the main groups of aceto-

clastic methanogens: *Methanosarcina* dominates with significant concentrations of acetate, and *Methanosaeta* dominates with small concentrations of acetate);

ρ_{mSar}, ρ_{mSae} — are the maximum specific rates of conversion of acetate to methane by two groups of acetoclastic methanogens, respectively;

K_{mSar}, K_{mSae} — the corresponding half-saturation constants for the acetate concentration. For hydrogenotrophic methanogenesis, we get

$$\rho_{meth}^{H_2/CO_2} = \rho_{m\,meth}^{H_2/CO_2} B_{H_2-meth} \frac{H_2}{K_{H_2}+H_2} \frac{CO_2}{K_{CO_2}+CO_2}, \quad (14)$$

where H_2 and CO_2 — are the concentrations of hydrogen and carbon dioxide, respectively;

B_{H_2-meth} is the concentration of hydrogenotrophic methanogens;

$\rho_{m\,meth}^{H_2/CO_2}$ is the maximum specific rate of $H_2 + CO_2$ transformation to methane by hydrogenotrophic methanogens;

K_{CO_2}, K_{HO_2} are the half-saturation constants for carbon dioxide and hydrogen.

To ensure the overall material balance (with respect to carbon) in the formation of methane from cellulose, it is necessary to take into account the decomposition of inactive biomass and its further transformation as a result of lysis back into acetate, carbon dioxide and hydrogen:

$$C_5H_7NO_2 + \frac{14}{3}H_2O \rightarrow$$
$$\rightarrow \frac{5}{3}CH_3COOH + \frac{5}{3}CO_2 + \frac{10}{3}H_2 + NH_3. \quad (15)$$

As a result, taking into account the stoichiometric equations (5), (10), (11), (15), the formation of methane from cellulose occurring in water is described by the following system of differential equations:

$$\frac{dCel}{dt} = -k_h Cel,$$
$$\frac{dAc}{dt} = 2k_h Cel + \frac{5}{3}k_{hB} B_{nv} - \rho_{Sar} - \rho_{Sae},$$
$$\frac{dH_2}{dt} = 4k_h Cel + \frac{10}{3}k_{hB} B_{nv} - (4 - 10Y_{H_2/CO_2})\rho_{meth}^{H_2/CO_2},$$
$$\frac{dCO_2}{dt} = 2k_h Cel + \frac{5}{3}k_{hB} B_{nv} + (1 - 2.5Y_{Sar})\rho_{Sar} + (1 - 2.5Y_{Sae})\rho_{Sae} - \rho_{meth}^{H_2/CO_2},$$
$$\frac{dCH_4}{dt} = (1 - 2.5Y_{Sar})\rho_{Sar} + (1 - 2.5Y_{Sae})\rho_{Sae} + (1 - 5Y_{H_2/CO_2})\rho_{meth}^{H_2/CO_2},$$
$$\frac{dB_{Sar}}{dt} = Y_{Sar}\rho_{Sar} - k_{dSar}B_{Sar},$$
$$\frac{dB_{Sae}}{dt} = Y_{Sae}\rho_{Sae} - k_{dSae}B_{Sae},$$
$$\frac{dB_{H_2-meth}}{dt} = Y_{H_2/CO_2}\rho_{meth}^{H_2/CO_2} - k_{dH_2-meth}B_{H_2-meth},$$
$$\frac{dB_{nv}}{dt} = -k_{hBnv}B_{nv} + k_{dSar}B_{Sar} + k_{dSae}B_{Sae} + k_{dH_2-meth}B_{H_2-meth}, \quad (16)$$

where *Cel*, *Ac*, CH_4, H_2 and CO_2 are the concentrations of cellulose, acetate, dissolved methane, hydrogen, and carbon dioxide, respectively;

t is time;

k_h is the kinetic reaction constant of the 1st order for the hydrolysis of cellulose, B_{nv} is the inactive biomass concentration;

k_{hBnv} is the 1st order rate constant in the process of lysis of inactive biomass;

k_{dSar}, k_{dSae}, k_{dH_2-meth} are the corresponding biomass decay constants;

Y_{Sar}, Y_{Sae}, Y_{dH_2/CO_2} are the corresponding biomass yield coefficients for the acetoclastic methanogens *Methanosarcina, Methanosaeta* and hydrogenotrophic methanogens. In the model (16), the total carbon balance is maintained.

Along with the traditional variables contained in system (16), the model additionally included variables containing the isotope ^{13}C:

$$\frac{d\,{}^{13}Cel}{dt} = -\frac{{}^{13}Cel}{Cel}\frac{1}{\alpha_{Cel}}k_h Cel,$$
$$\frac{{}^{13}Ac}{dt} = -\frac{{}^{13}Ac}{Ac}\left(\frac{1}{\alpha_{AcOx}}\alpha_{AcOx} + \frac{1}{\alpha_{Sar}}\alpha_{Sar} + \frac{1}{\alpha_{Sae}}\alpha_{Sae}\right) + 2\frac{{}^{13}Cel}{Cel}\frac{1}{\alpha_{Cel}}k_h Cel + \frac{5}{3}\frac{{}^{13}B_{nv}}{B_{nv}}\frac{1}{\alpha_{B_{nv}}}k_{hB}B_{nv},$$
$$\frac{d\,{}^{13}CO_2}{dt} = 2\frac{{}^{13}Cel}{Cel}\frac{1}{\alpha_{Cel}}k_h Cel + \frac{{}^{13}Ac}{Ac}\left(\frac{1}{\alpha_{Sar}}(1 - 2.5Y_{Sar})\alpha_{Sar} + \frac{1}{\alpha_{Sae}}(1 - 2.5Y_{Sae})\alpha_{Sae} + (2 - 5Y_{AcOx})\frac{1}{\alpha_{AcOx}}\alpha_{AcOx}\right) - \frac{{}^{13}CO_2}{CO_2}\frac{1}{\alpha_{H_2/CO_2}}\alpha_{meth}^{H_2/CO_2} + \frac{5}{3}\frac{{}^{13}B_{nv}}{B_{nv}}\frac{1}{\alpha_{B_{nv}}}k_{hB}B_{nv},$$

$$\frac{d\,^{13C}CH_4}{dt} = \frac{^{13C}Ac}{Ac}\left(\frac{1}{\alpha_{Sar}}(1-2.5\,Y_{Sar})\,\alpha_{Sar} + \frac{1}{\alpha_{Sae}}(1-2.5\,Y_{Sae})\,\alpha_{Sae}\right) + \frac{^{13}CO_2}{CO_2}\frac{1}{\alpha_{H_2/CO_2}}(1-5\,Y_{H_2/CO_2})\,\alpha_{meth}^{H_2/CO_2},$$

$$\frac{d\,^{13C}B_{Sar}}{dt} = \frac{^{13C}Ac}{Ac}\frac{1}{\alpha_{Sar}}Y_{Sar}\alpha_{Sar} - k_{d\,Sar}\,^{13C}B_{Sar},$$

$$\frac{d\,^{13C}B_{Sae}}{dt} = \frac{^{13C}Ac}{Ac}\frac{1}{\alpha_{Sae}}Y_{Sae}\alpha_{Sae} - k_{d\,Sae}\,^{13C}B_{Sae},$$

$$\frac{d\,^{13C}B_{AcOx}}{dt} = \frac{^{13C}Ac}{Ac}\frac{1}{\alpha_{AcOx}}Y_{AcOx}\alpha_{AcOx} - k_{d\,AcOx}\,^{13C}B_{AcOx},$$

$$\frac{d\,^{13C}B_{H_2/CO_2}}{dt} = \frac{^{13}CO_2}{CO_2}\frac{1}{\alpha_{H_2/CO_2}}Y_{H_2/CO_2}\alpha_{meth}^{H_2/CO_2} - k_{d\,H_2/CO_2}\,^{13C}B_{H_2/CO_2},$$

$$\frac{d\,^{13C}B_{nv}}{dt} = -\frac{^{13C}B_{nv}}{B_{nv}}\frac{1}{\alpha_{B_{nv}}}k_{hB}B_{nv} + k_{d\,Sar}\,^{13C}B_{Sar} + k_{d\,Sae}\,^{13C}B_{Sae} + k_{d\,AcOx}\,^{13C}B_{AcOx} + k_{d\,H_2/CO_2}\,^{13C}B_{H_2/CO_2}, \quad (17)$$

where $^{13C}B_{Sar}$, $^{13C}B_{Sae}$, $^{13C}B_{H_2-meth}$ and $^{13C}B_{nv}$ are the concentrations of the 'heavier' biomass of *Methanosarcina*, *Methanosaeta* acetoclastic methanogens, hydrogenotrophic methanogens and inactive biomass containing ^{13}C, respectively; α_{Cel}, α_{Sar}, α_{Sae}, α_{AcOx}, α_{H_2/CO_2}, α_{Bn_v} are the carbon isotope fractionation coefficients in the process of hydrolysis and acidogenesis, acetoclastic methanogenesis, hydrogenotrophic methanogenesis and decomposition of inactive biomass, respectively.

Note that in the model combining (16) and (17), the balance of both total carbon (C) and its isotope (^{13}C) is observed, which allows us to correctly describe their dynamics.

Model of anaerobic oxidation of methane by nitrate

For the mathematical description of DAOM, the following stoichiometric reaction was used:

$$CH_4 + \frac{8-23Y}{5}NO_3^- = Y\,C_5H_7NO_2 + \frac{8-28Y}{10}N_2 + (1-5Y)CO_2 + \frac{8-23Y}{5}OH^- + \frac{6}{5}(1-Y)H_2O. \quad (18)$$

The Monod function for AOM with two substrates limiting the total rate (dissolved methane and nitrate) was used to describe the growth of biomass, consumption of the substrate and product formation:

$$\rho_{AOM} = \rho_{m\,AOM}B_{AOM} \times \frac{CH_4}{K_{CH_4} + CH_4}\frac{NO_3^-}{K_{NO_3^-} + NO_3^-}, \quad (19)$$

where B_{AOM} is the biomass concentration of methane-oxidizing microorganisms; CH_4, NO_3^- are the concentrations of dissolved methane and nitrate ion, respectively; ρ_{AOM} is the methane consumption rate; ρ_{mAOM} is the maximum specific rate of methane consumption; K_{CH_4}, $K_{NO_3^-}$ are the corresponding half-saturation con stants. As a result, the DAOM process was described by the following system of equations:

$$\frac{dCH_4}{dt} = -\rho_{AOM},$$

$$\frac{dNO_3^-}{dt} = -\frac{8-23Y_{AOM}}{5}\rho_{AOM},$$

$$\frac{dCO_2}{dt} = (1-5Y_{AOM})\,\rho_{AOM},$$

$$\frac{dN_2}{dt} = \frac{8-23Y_{AOM}}{10}\,\rho_{AOM},$$

$$\frac{dB_{AOM}}{dt} = Y_{AOM}\,\rho_{AOM} - k_d\,B_{AOM}, \quad (20)$$

where N_2, CO_2 are the dissolved nitrogen and carbon dioxide concentrations, respectively; Y_{AOM} is the economic coefficient, reflecting the share of the substrate, turning into the biomass of methane-oxidizing microorganisms.

The change in the concentration of dissolved ^{13}C-CH_4 as a result of microbiological reactions was described by the following equation:

$$\frac{d\,^{13}C\text{-}CH_4}{dt} = \frac{^{13}C\text{-}Ac}{Ac}\frac{1}{\alpha_{Acetocl}}(1-2.5\,Y_{Acetocl})\,\rho_{Acetocl} + \frac{^{13}C\text{-}CO_2}{CO_2}\frac{1}{\alpha_{H_2/CO_2-meth}}(1-5\,Y_{H_2/CO_2-meth})\,\rho_{meth}^{H_2/CO_2} - \frac{^{13}C\text{-}CH_4}{CH_4}\frac{1}{\alpha_{AOM}}\rho_{AOM}. \quad (21)$$

From (21) it thus follows that the content of ^{13}C in methane depends on the rates of acetoclastic and hydrogenotrophic methanogenesis, as well as on the rate of the DAOM process. Moreover, according to (21), the contribution of these pro-

cesses has different signs (in the process of methanogenesis, methane is the product of the reaction, and in the process of DAOM it is the substrate of the reaction). In the mathematical model, the mass exchange of CH_4, CO_2 и H_2 between the liquid and gaseous phases [Vavilin et al., 2018a] was also taken into account:

$$\frac{dP_{CO_2}}{dt} = -K_{P\,CO_2}\left(k_{H\,CO_2}P_{CO_2} - CO_{2\,dis}\right)\frac{V_L}{V_G}\frac{V_M}{1000},$$

$$\frac{dP_{CH_4}}{dt} = -K_{P\,CH_4}\left(k_{H\,CH_4}P_{CH_4} - CH_{4dis}\right)\frac{V_L}{V_G}\frac{V_M}{1000},$$

$$\frac{dP_{H_2}}{dt} = -K_{P\,H_2}\left(k_{H\,H_2}P_{H_2} - H_{2dis}\right)\frac{V_L}{V_G}\frac{V_M}{1000}, \qquad (22)$$

where K_P is the corresponding mass transfer constants [1 / day] determining how rapidly the concentration of the dissolved gas approaches an equilibrium value, these constants were considered equal for all gases;

$k_{H\,CO_2}$, $k_{H\,CH_4}$, $k_{H\,H_2}$ are the Henry constants (35, 1.36 and 0.87 mmol / l bar) for CO_2, CH_4 and H_2, respectively;

P_{CO_2}, P_{CH_4}, P_{H_2} are the partial pressures CO_2, CH_4, and H_2; CO_{2dis}, CH_{4dis} and H_{2dis} are the dissolved gas concentrations;

V_L/V_G is the ratio of the volumes of the liquid and gas phase in the incubation vessel;

V_M is the volume of one mole of gas in ml.

Additionally, mass transfer between the dissolved and gaseous forms of nitrogen N_2 was considered.

To describe the mass transfer of gases containing the ^{13}C isotope ($^{13}CO_2$ and $^{13}CH_4$), the following equations were considered:

$$\frac{dP_{^{13}CO_2}}{dt} = -\frac{1}{\alpha_{ex\,CO_2}}K_L \times$$
$$\times\left(k_{H\,CO_2}P_{^{13}CO_2-gas} - {}^{13}CO_{2\,dis}\right)\frac{V_L}{V_G}\frac{V_M}{1000},$$

$$\frac{dP_{^{13}CH_4}}{dt} = -\frac{1}{\alpha_{ex\,CH_4}}K_L \times$$
$$\times\left(k_{H\,CH_4}P_{^{13}CH_4-gas} - {}^{13}CH_{4\,dis}\right)\frac{V_L}{V_G}\frac{V_M}{1000}, \qquad (23)$$

where α_{ex} is the fractionation coefficient in the process of mass exchange of $^{13}CO_2$ and $^{13}CH_4$ in the liquid and gas phases. In the systems of equations (22) and (23), the variables CO_{2dis}, CH_{4dis}, $^{13}CO_{2dis}$, $^{13}CH_{4dis}$ mean concentrations of dissolved gases and their isotopes.

The ratio of ^{13}C and ^{12}C isotopes in cellulose, methane, carbon dioxide, acetate, and biomass was calculated by the formula:

$$\delta^{13}Q\,[‰] = \left[\frac{^{13}Q/(Q - {}^{13}Q)}{0.0112372} - 1\right], \qquad (24)$$

where ^{13}Q is the concentration of enriched ^{13}C substrates, products and biomass; Q — concentration of components without consideration of their isotopic composition.

The contribution of hydrogenotrophic methanogenesis to the total methane production was calculated on the basis of the ratio:

$$f_C = \frac{CH_4^{H_2/CO_2}}{CH_4^{H_2/CO_2} + CH_4^{Acetate}} - 1, \qquad (25)$$

where $CH_4^{H_2/CO_2}$ and $CH_4^{Acetate}$ is the amount of methane formed from $H_2 + CO_2$ (11) and acetate (10), respectively.

To solve the system of differential equations presented above, the ode15s solver was used, which implemented a multi-step method of numerical integration of variable order (5 order was used) of the MATLAB system [MathWorks Inc., 1984]. Valid values of relative (10^{-14}) and absolute (10^{-16}) errors were specified. The solver was used in the mode of solving a system of differential equations written explicitly.

The model calibration consisted of two stages. At the first stage, only experimental data were used for the generated methane without considering its isotopic composition. Note that the coefficients found of microbial biomass growth and decay are not unequivocal, since microbial biomass concentrations are usually not measured, and the substrate utilization rates (12)–(14), (19) depend primarily on the biomass concentration and maximum specific rate of substrate consumption.

In this case, the higher the initial biomass concentration B_0, the lower the value should be chosen for the maximum specific substrate consumption rate ρ_m. Kinetic coefficients characteristic of anaerobic microbiological processes are contained, in particular, in a summary paper [Batstone et al., 2002]. The key parameters used in the proposed model are listed in Table 1. In the model, the concentrations were calculated in moles, then they were recalculated to g / l. At the second calibration stage, $\delta^{13}CH_4$ experimental data were used. As a result, the isotope fractionation coefficients α were determined.

The article is supplemented with two files (MATLAB): 1). the main ("main") with a graphical representation of the results of calculations and 2). a subprogram ("equations") containing the equations themselves. This allows the reader to directly analyze the dynamics of the DAOM process. It also contains the values of all parameters and the initial conditions of the variables of the model. The total number of model variables, including isotopic

Table 1 / Таблица 1

Key parameters ($\propto_m$, ρ_m, K_s) and initial biomass concentrations B_o in the mathematical model describing the formati on and anaerobic oxidation of methane in incubations with minerotrophic peat. In the simulations for the concentrations, mol / l units were used in accordance with the experimental data, then they were converted into weight units

Ключевые параметры ($\propto_m$, ρ_m, K_s) и начальные концентрации биомассы Во математической модели, описывающие образование и анаэробное окисление метана в инкубациях с минеротрофным торфом. При моделировании для концентраций использовались единицы мол/л. В соответствии с экспериментальными данными, затем они пересчитывались в весовые единицы

Process	B_0, g/l	*rate constants k_h, day^{-1} $\mu_m = \rho_m Y$, day^{-1}	K_s, mg/l	**α_C
Hydrolysis and acidogenesis of cellulose		$k_h = 0.012$		1.001
Acetoclastic methanogenesis ***	7.3×10^{-3}	0.02	0.2	1.01
Hydrogenotrophic methanogenesis	1.1×10^{-3}	0.1075	2×10^{-8} (H_2)	1.075
DAOM	1.4×10^{-2}	0.014	1.6×10^{-3} (CH_4)	1.018

*The model for all groups of biomass used the same biomass yield coefficients Y, equal to 0.025 *mol mol*$^{-1}$ and the biomass decomposition coefficient k_d, equal $0.04\propto_m$;
**The last column indicates carbon isotope fractionation factors (α_C) for individual processes of hydrolysis / acidogenesis, acetoclastic and hydrogenotrophic methanogenesis and anaerobic oxidation of methane, respectively;
***For calculations, only one group of acetoclastic methanogens was assumed in the model.

variables, is 30. The total number of parameters, including the initial concentrations of the variables is as high as 70. The dimensions of the variables and parameters of the dynamic model, close to that used in this article, were given in a recently published article [Vavilin et al., 2018b]. The matrix form of the model is also presented there.

RESULTS AND DISCUSSION

The dynamics of microbiological processes is presented in Figure 2. Cellulose decomposition, described by a simple first-order reaction, occurs slowly (Fig. 2a). Since the biomass concentrations of acetoclastic methanogens and hydrogenotrophic methanogens are significant (Fig. 2e), the current concentrations of acetate and dissolved hydrogen are small (Fig. 2a, 2c). In this case, the rate of formation of methane is determined by the rate of hydrolysis of cellulose. In the absence of nitrate, the concentration of dissolved methane increases linearly (Fig. 2c), respectively, and the pressure of methane increases linearly (Fig. 2g). Since methane in this case is a reaction product, the value decreases (Fig. 2h), due to the fact that heavier carbon remains in the substrate (cellulose) and partially passes into the biomass of microorganisms (not shown in Fig. 2). The proportion of hydrogenotrophic methanogenesis in the formation of methane at the end of the process is close to the classical ratio of 1/3 (Fig. 2i), since the model did not take into account such processes as syntrophic acetate oxidation and homoacetogenesis.

The introduction of nitrate leads to the dominance of the reaction rate of DAOM (18) over the rate of the multistep process of methane formation from cellulose. The nitrate concentration in the time interval under study decreases linearly, while the partial pressure of methane and the concentration of dissolved methane decrease slowly (Fig. 2d, 2g, 2c). Since methane in the process of its anaerobic oxidation with nitrate is a substrate, the value $\delta^{13}CH_4$ increases (Fig. 2h). According to (19), the rate of DAOM decreases with a decrease in the concentration of nitrate, which leads to a slower process of fractionation and a $\delta^{13}CH_4$ increase (Fig. 2h). The value of the apparent fractionation coefficient α_C (8) decreases (Fig. 2f). Thus, according to the results of incubation experiments and the calculation of the apparent fractionation coefficient, it is possible to determine whether the process of anaerobic oxidation of methane influences its formation. The carbon isotope fractionation rate in the process of methane oxidation with nitrate is 1.018, which is comparable to the carbon isotope fractionation rate in the process of acetoclastic methanogenesis, equal to 1.01 [Penning et al., 2006]. At the same time, the fractionation factor of carbon isotopes in the process of hydrogenotrophic methanogenesis is much larger (1.075) [Whiticar, 1999] than that of acetoclastic methanogenesis.

As noted above, the dynamic model used has a large number of variables and parameters. The model was calibrated in 2 stages. At the same time, at the 1st stage, the model was calibrated without considering the isotopic composition of the model variables. The values of isotopic variables are about 1% of the values of ordinary variables. According to the dynamic model, the assessment of changes in isotopic variables allows us to com-

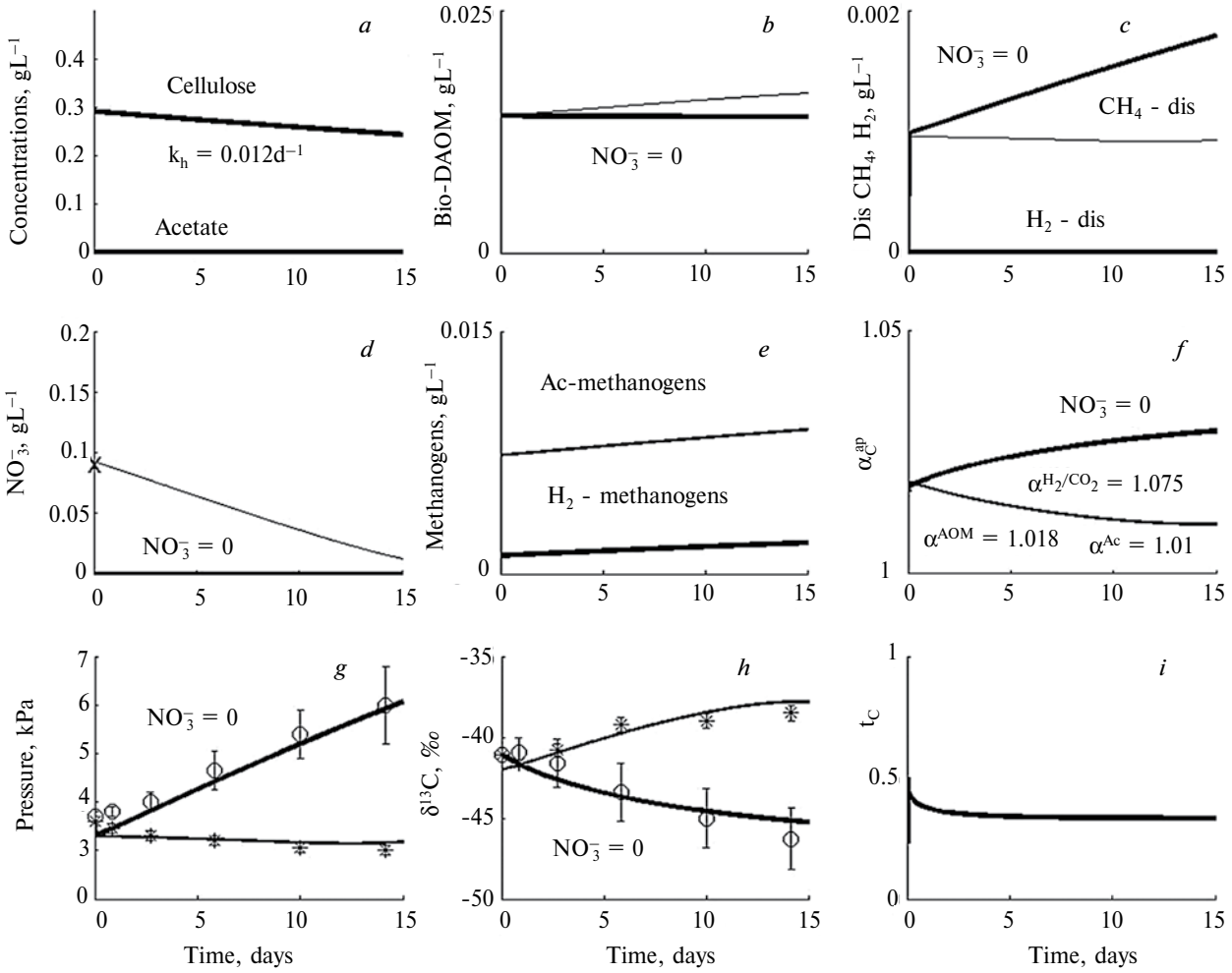

Fig. 2. Dynamics of anaerobic oxidation with methane nitrate. Symbols: experiment [Smemo, Yavitt, 2007]; curves: dynamic model in the absence and presence of nitrate. Key kinetic coefficients are presented in Table 1. Thick lines show the system dynamics in the absence of nitrate (Fig. 2b, 2c, 2f, 2g and 2h). It is assumed that the conversion of cellulose to methane is the same in the presence and absence of nitrate (Fig. 2a, 2e, 2i).

Legend: 'a': cellulose and acetate concentrations; 'b': biomass concentration of anaerobic methane-oxidizing microorganisms; 'c': the concentration of dissolved methane and hydrogen; 'd': nitrate concentration; 'e': concentration of biomass of acetoclastic and hydrogenotrophic methanogens; 'f': apparent stable carbon fractionation ratio (8); 'g': methane partial pressure; 'h': the ratio of ^{13}C and ^{12}C isotopes in methane in ppm (1); 'i': the proportion of hydrogenotrophic methanogenesis in the total methane production (25)

Рис. 2. Динамика анаэробного окисления метана нитратом. Символы: эксперимент [Smemo, Yavitt, 2007]; кривые: динамическая модель в отсутствии и присутствии нитрата. Ключевые кинетические коэффициенты представлены в Табл. 1. Толстыми линиями показана динамика системы в отсутствии нитрата (рис. 2b, 2c, 2f, 2g и 2h). Принимается, что превращение целлюлозы в метан идет одинаково в присутствии и отсутствии нитрата (рис. 2a, 2e, 2i).

Условные обозначения: 'a': концентрации целлюлозы и ацетата; 'b': концентрация биомассы анаэробных метанокисляющих микроорганизмов; 'c': концентрация растворенного метана и водорода; 'd': концентрация нитрата; 'e': концентрация биомассы ацетокластических и водородотрофных метаногенов; 'f': кажущийся коэффициент фракционирования стабильного углерода (8); 'g': парциальное давление метана; 'h': отношение изотопов ^{13}C и ^{12}C в метане в промилях (1); 'i': доля водородотрофного метаногенеза в общей продукции метана (25)

pare the dominance of various processes (in our case, the processes of anaerobic methane formation and its oxidation). In accordance with (21), the change in the concentration of heavier methane $^{13}CH_4$ depends on the fractionation coefficients of carbon isotopes in the formation of methane and its oxidation. The model showed that, when describing the $^{13}CH_4$ dynamics, the most sensitive parameter is the fractionation coefficient in the DAOM process (Fig. 3). From Fig. 3, it follows that for the coefficient the third digit after the point is significant. We emphasize once again that the change in the reaction rate through the kinetic isotope effect is small.

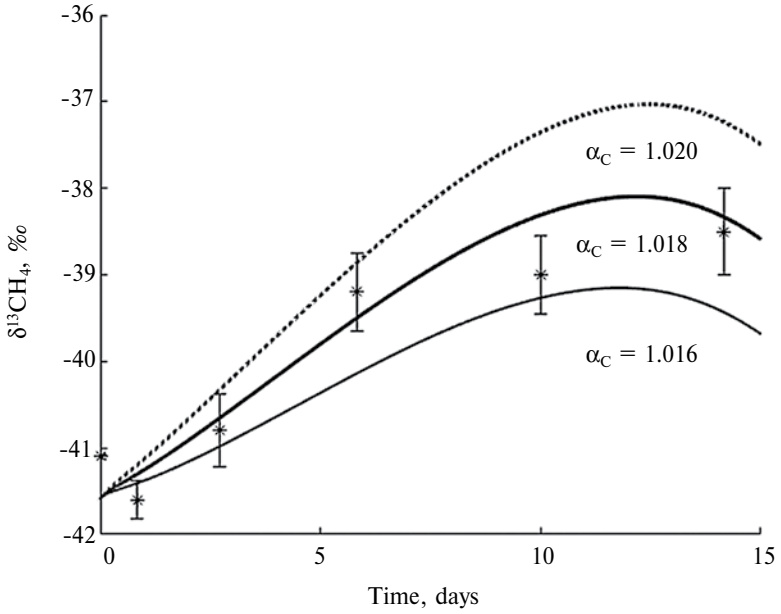

Fig. 3. The dynamics of the $\delta^{13}C\text{-}CH_4$ value at different values of the fractionation factor α_C^{DAOM}
Рис. 3. Динамика величины $\delta^{13}C\text{-}CH_4$ при разных значениях коэффициента фракционирования α_C^{DAOM}

According to the model, an increase in the initial concentration of nitrate increases the rate of DAOM, which leads to a decrease in the concentration of dissolved methane and the partial pressure of methane. The $\delta^{13}CH_4$ value increases. During incubation, the concentration of dissolved nitrate limits the overall rate of the DAOM process. The nitrate concentration decreases linearly (Fig. 2d), and the concentration of dissolved methane varies slightly (Fig. 2c). Only at the end of incubation with a small concentration of nitrate, the rate of formation of methane from cellulose begins to prevail over the rate of methane consumption as a result of DAOM.

In this case, the partial pressure of methane, and, accordingly, the concentration of dissolved methane begins to increase (Fig. 2g, 2c). The $\delta^{13}CH_4$ value begins to decrease (Fig. 2h). In field studies, Shi et al. [Shi et al., 2017] showed that the presence of DAOM in peat bogs penetrated

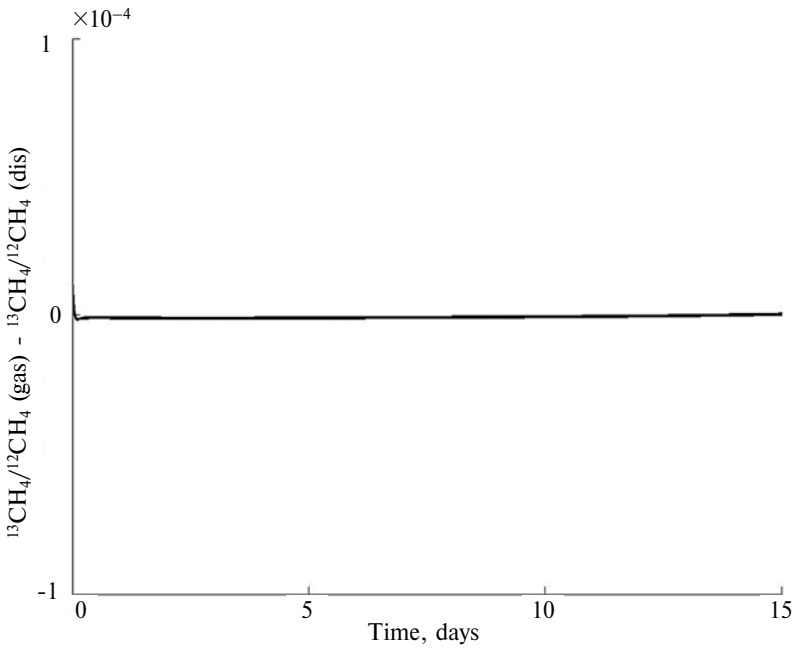

Fig. 4. The ratio of ^{13}C and ^{12}C isotopes for dissolved and gaseous methane in an incubation experiment
Рис. 4. Отношение изотопов ^{13}C и ^{12}C для растворенного и газообразного метана в инкубационном эксперименте

by fertilizers can be controlled by the amount of nitrate used and by the depth of its penetration into the oxygen-free layer.

According to Knox et al. [Knox et al., 1992], the ratio of $^{13}C/^{12}C$ isotopes in dissolved and gaseous methane is almost the same. The mathematical model confirmed this (Fig. 4). In equations (23), the fractionation factor $\alpha_{ex\,CH_4}$ in the mass exchange process was small (0.001). Consequently, the main fractionation of carbon isotopes occurs as a result of microbiological processes, primarily in the DAOM process.

Earlier, Smemo and Yavitt [Smemo, Yavitt, 2007] in their calculations used a model assuming a=mixing of methane, added at the beginning of the process with biologically formed methane. It was assumed that during the anaerobic oxidation of methane with nitrate, the fractionation of carbon isotopes does not occur. Using the experimental data from Smemo and Yavitta, an approximate estimate of the fractionation carried out by us using a dynamic model showed that the fractional fraction of carbon isotopes in the process of oxidation of methane with nitrate is significant (1.018). According to Vavilin and Rytov [2016], the coefficient of fractionation of carbon isotopes during the oxidation of methane with nitrite was 1.032.

CONCLUSIONS

In the course of simultaneous formation of methane from cellulose and its anaerobic oxidation with nitrate, the anaerobic oxidation of methane prevails. During incubation, the concentration of dissolved nitrate limits the overall rate of the DAOM process. Since methane for DAOM is a substrate, methane is enriched with heavier ^{13}C carbon, which leads to an increase of $\delta^{13}C\text{-}CH_4$. In contrast to [Smemo, Yavitt, 2007], an estimate of carbon isotope fractionation using a dynamic model showed that the fractionation factor of carbon isotopes during the methane oxidation by nitrate is significant (1.018) and comparable to the fractionation factor for acetoclastic methanogenesis (1.01) used in the calculations. It is much less than the corresponding fractionation coefficient for hydrogenotrophic methanogenesis (1.075). From incubation experiments and calculation of the apparent fractionation factor, it is possible to determine whether the process of anaerobic oxidation of methane influences its formation.

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