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KINETIC ISOTOPIC EFFECT: STATIC RAYLEIGH EQUATION AND BASIC DYNAMIC ISOTOPE EQUATION FOR THE SUBSTRATE IN THE DESCRIPTION OF NITRITE-DEPENDENT ANAEROBIC OXIDATION OF METHANE

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The article analyzes the results of modeling the dynamics of nitrite-dependent methane oxidation (N-DAMO) by *Methylomirabilis oxyfera* microorganisms using the standard isotope dynamic equations. Without specifying a specific function of the rate of the process, the traditional static Rayleigh equation is derived from the basic dynamic isotope equation. Thus, the equation of the 1st order in terms of the substrate is only a special case in the derivation of the Rayleigh equation. It was shown that the dominant fractionation of carbon isotopes occurs in the process of the microbiological reaction of anaerobic oxidation of methane by nitrite, and not in the physical process of mass transfer of dissolved methane into the gas phase. In contrast to the static Rayleigh equation, the dynamic description of the process of fractionation of stable isotopes is important when describing the parallel transformations of the substrate.

Key words: kinetic isotope effect, static Rayleigh equation, basic dynamic isotope equation, N-DAMO process
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INTRODUCTION

The carbon dioxide and methane are the major greenhouse gases and knowledge about mechanisms effecting their formation are obligated for the ability of conversion of local emissions evaluation to the atmosphere to the global one [Bridgeham et al., 2013]. The ration of stable carbon isotopes $^{13}C/^{12}C$ in methane and carbon dioxide may indicate climatic changes of the Earth [Hornibrook et al., 2000; Elsig et al., 2009; Bauska et al., 2016]. Two simultaneously behaving processes: the formation and the oxidation of methane determine the total of $\delta^{13}C-CH_4$. Herewith, the value $\delta^{13}C-CO_2$ is playing an essential role.

In the isotope researches two types of isotopic effects are usually considered: thermodynamic and kinetic [Galimov, 1973]. A separation of isotopes in equilibrium conditions which was caused by the differences in energetic states of molecules that contain different isotopes is named the thermodynamic isotope effect. The kinetic isotopic effect consists in changing the rate of chemical, biological and physical processes when replacing an atom in a reacting substance molecule with its isotope.

The approach presented below is based on the equations of formal chemical kinetics. Modeling with standard isotopic dynamic equations is based on a joint description of processes in which isotopic equations are not considered and specific isotope equations. The proposed in this paper N-DAMO model showed that the dominant fractionation of carbon isotopes occur during the microbiological reaction of methane anaerobic oxidation by nitrite not during the physical process of mass-transfer of gaseous methane to water.

THE RAYLEIGH EQUATION

In vivo, the ratio of stable carbon isotopes $^{13}C/^{12}C$ is a small value, about 0.01; in this case it is traditionally determined by the difference of the ratio $^{13}C/^{12}C$ in the study sample and the standard which are expressed in permilles (thousandths). As a standard of Pee Dee Belemnite (PDB) is given the ratio $^{13}C/^{12}C$ in *Belemnitella americana* shell fossil from the Pee D Formation (South Carolina, USA) which is characterized by a high content of ^{13}C [Craig,1957; Galimov,1968]:

$$\delta^{13}C(o/oo)=\left[\frac{\left(^{13}C/^{12}C\right)_{обр}}{\left(^{13}C/^{12}C\right)_{PDB}}-1\right]\times 1000 \tag{1}$$

where the $\delta^{13}C$ value is named isotopic signature. The concentration differences of heavier isotopes in the substrate during chemical-biological transformations are generally small.

The Rayleigh equation [1896], originally proposed to describe the diffusion of gases, is commonly used to calculate the fractionation (separation) factor of stable isotopes [Mariotti et al., 1981; Hornibrook et al., 2000; Holler et al., 2009; Galand et al., 2010; Conrad et al., 2011]:

$$R_t/R_0=\left(S_t/S_0\right)^{(1/\alpha-1)} \tag{2}$$

where R_0 and R_t – the ratio of heavy and light stable isotopes in the substrate at time 0 and t, respectively; S_0 and S_t - input and instant concentrations of the substrate; α –the fractionation factor of stable isotopes between the substrate and product in closed completely stirred system. The value α is determined from the inclination of a straight line [Mariotti et al., 1981] obtained in double logarithmical coordinates following from the Rayleigh equation:

$$\ln \frac{\delta_t + 1000}{\delta_0 + 1000} = (1/\alpha - 1)\ln\left(\frac{S_t}{S_0}\right) \tag{3}$$

where δ_0 and δ_t correspond to the reaction beginning $t = 0$ and instant time t with characteristic values of the fractionation factors of stable isotopes α.

Consider next a set of two 1st-order equations for the substrate concentration for lighter ${}^{l}S$ and heavier ${}^{h}S$ substrates:

$$\begin{cases} \frac{d\,{}^{l}S}{dt} = -k \cdot {}^{l}S \\ \frac{d\,{}^{h}S}{dt} = -\frac{1}{\alpha} k \cdot {}^{h}S \end{cases} \tag{4}$$

where rate constant of the 1st-order substrate reaction with heavier isotope $k_h = k/\alpha$ $(\alpha > 1)$ is something less than for the combined substrate where the isotopes are not differentiated.

The solutions to both equations are the following:

$$\begin{cases} {}^{l}S_t = {}^{l}S_0 \cdot e^{-k \cdot t} \quad \text{или} \quad \ln\left({}^{l}S_t / {}^{l}S_0\right) = -k \cdot t \\ {}^{h}S_t = {}^{h}S_0 \cdot e^{-k/\alpha \cdot t} \quad \text{или} \quad \ln\left({}^{h}S_t / {}^{h}S_0\right) = -k/\alpha \cdot t \end{cases} \tag{5}$$

Dividing the 1st equation by the 2nd equation, for the isotope fractionation factor α we obtain:

$$\frac{\ln\left({}^{l}S_t / {}^{l}S_0\right)}{\ln\left({}^{h}S_t / {}^{h}S_0\right)} = \alpha \tag{6}$$

Therefore

$$\ln\left({}^{h}S_t / {}^{h}S_0\right)^{\alpha} = \ln\left({}^{l}S_t / {}^{l}S_0\right) \tag{7}$$

Or

$${}^{h}S_t / {}^{h}S_0 = \left({}^{l}S_t / {}^{l}S_0\right)^{1/\alpha} \tag{8}$$

Dividing right and left parts of the equation by ${}^{l}S_t / {}^{l}S_0$ and by taking the lighter and combined substrates concentrations ${}^{l}S \approx S = {}^{l}S + {}^{h}S$ are equal with great accuracy we find classical Rayleigh equation (2):

$$\frac{\frac{{}^{h}S_t}{{}^{l}S_t}}{\frac{{}^{h}S_0}{{}^{l}S_0}} = \left(\frac{S_t}{S_0}\right)^{\left(\frac{1}{\alpha} - 1\right)} \tag{9}$$

To date, the Rayleigh equation (2) has been cited thousands of times in the isotope literature. Frequently, the citation of the paper itself doesn't occur while the name of Rayleigh is mentioned. In order to describe the isotopic effect, 1st-order chemical kinetics in terms of the substrate *S* concentration is usually assumed, where the kinetic coefficient is lower for a substrate with a heavier isotope. Traditionally, the 1st-order equation justifies the applicability of the Rayleigh equation. The fractionation factor α may be easily defined with LR (linear regression) (3). Therewith the fraction factor is considered to be constant. Meanwhile, this value may not characterize the study process. Thus, Holler and colleagues [Holler et al, 2009] came to a conclusion that a satisfactory explanation for the significant range of the fractionation factors of stable isotopes in various sediments of the seas doesn't exist (carbon: 1,012 - 1,039; hydrogen: 1,109 - 1,315). In fact, the traditional Rayleigh equation is static, since it defines the substrate concentration only at times 0 and *t* and does not depend on the dynamics of the substrate concentration in the time interval from 0 to *t*.

THE BASIC DYNAMIC ISOTOPIC EQUATION

The basic isotopic equation is written in the following way [Vavilin et al., 2014; Vavilin, Rytov, 2015]:

$$\frac{d\,{}^{h}S}{dt} = \frac{1}{\alpha}\frac{{}^{h}S}{S}\frac{dS}{dt} \qquad (10)$$

where ${}^{h}S$ and $S = {}^{l}S + {}^{h}S \approx {}^{l}S$ - concentrations of heavier and combined substrates, respectively; $\frac{dS}{dt}$ - the rate of change in the combined substrate concentration. The non-linear function as Monod is usually used for describing the rate of biological process with one or two substrates.

The value α which is taken from the basic isotopic equation may be written as follows:

$$\frac{\frac{dS}{dt}\Big/S}{\frac{d\,{}^{h}S}{dt}\Big/{}^{h}S} = \alpha \qquad (11)$$

Or

$$\frac{1}{\alpha}\frac{d\ln S}{dt} = \frac{d\ln {}^{h}S}{dt} \qquad (12)$$

After integrating in the limits from 0 to *t* we find:

$$\ln\left(S_t\right)^{1/\alpha} - \ln\left(S_0\right)^{1/\alpha} = \ln\left({}^{h}S_t\right) - \ln\left({}^{h}S_0\right) \qquad (13)$$

Or

$$\ln\left(\frac{S_t}{S_0}\right)^{1/\alpha} = \ln\left(\frac{{}^{h}S_t}{{}^{h}S_0}\right) \qquad (14)$$

i.e.

$$\left(\frac{S_t}{S_0}\right)^{1/\alpha} = \frac{{}^hS_t}{{}^hS_0} \tag{15}$$

Dividing right and left parts of the equation by ${}^lS_t \big/ {}^lS_0$ and by taking that the lighter and combined substrates concentrations with great accuracy are equal $({}^lS \approx S = {}^lS + {}^hS)$ we find. Thus, we find the classical Rayleigh equation (2) from the dynamic isotopic equation (10) without setting the specific function of the process rate $\frac{dS}{dt}$:

$$\frac{{}^hS_t \big/ {}^lS_t}{{}^hS_0 \big/ {}^lS_0} = \left(\frac{S_t}{S_0}\right)^{\frac{1}{\alpha}-1} \tag{16}$$

The equation (10) where variable refers to the substrate must be supplemented with isotopic equations for the reaction product (P) and the biomass (B) engaging the reaction itself:

$$\begin{aligned} \frac{d^hP}{dt} &= \frac{1}{\alpha}\frac{{}^hS}{S}\frac{dP}{dt} \\ \frac{d^hB}{dt} &= \frac{1}{\alpha}\frac{{}^hS}{S}\frac{dB}{dt} \end{aligned} \tag{17}$$

where ${}^hP$, ${}^hB$ и P , B, S - concentrations of product and biomass that contain heavier isotope and combined product, biomass and substrate, respectively.

It must be emphasized that the isotopic equations (10), (17) describe the dynamics of redistribution of the ratio of stable isotopes in the substrate, product and biomass during the formation of the reaction product (P) from the substrate (S) carried out by microorganisms (B). In the initial and final substrate, product and biomass, this ratio is different. It should be noted that the remaining CH_{4dis} substrate is "enriched” with the heavier isotope ^{13}C during the reaction.

DYNAMICAL MODEL OF NITRITE-DEPENDENT ANAEROBIC OXIDATION OF METHANE

Nitrite-dependent methane oxidation (N-DAMO) is characteristic for freshwater reservoirs [Deutzmann et al., 2014]. The general stoichiometric equation of anaerobic oxidation of methane with nitrite was considered in the article in the following form:

$$CH_4 + \left(\frac{8}{3} - \frac{20}{3}Y\right) HNO_2 + YNH_3 = YC_5H_7NO_2 + (1-5Y)CO_2 + \left(\frac{10}{3} - \frac{16}{3}Y\right) H_2O + \left(\frac{4}{3} - \frac{10}{3}Y\right) N_2 \tag{18}$$

Introducing the function $R = \rho_m B \frac{CH_{4dis}}{K_{SCH4} + CH_{4dis}} \frac{HNO_2}{K_{SHNO_2} + HNO_2}$, the dynamic model was written as the following system of differential equations:

$$\frac{dCH_{4dis}}{dt} = -R + K_L(SS - CH_{4dis})$$
$$\frac{dCH_{4gas}}{dt} = -K_L(SS - CH_{4dis})$$

$$\frac{d^{13}CH_{4dis}}{dt} = -\frac{1}{\alpha_{CB}}\frac{^{13}CH_{4dis}}{CH_{4dis}}R + \frac{1}{\alpha_g}K_L\left(^{13}SS - {}^{13}CH_{4dis}\right)$$
$$\frac{d^{13}CH_{4gas}}{dt} = -\frac{1}{\alpha_g}K_L\left(^{13}SS - {}^{13}CH_{4dis}\right)$$
$$\frac{dB}{dt} = YR$$
$$\frac{d^{13C}B}{dt} = \frac{1}{\alpha_{CB}}\frac{^{13}CH_{4dis}}{CH_{4dis}}YR$$
$$\frac{dCO_2}{dt} = (1-5Y)R$$
$$\frac{d^{13}CO_2}{dt} = \frac{1}{\alpha_{CB}}\frac{^{13}CH_{4dis}}{CH_{4dis}}(1-5Y)R$$
$$\frac{dHNO_2}{dt} = -\left(\frac{8}{3} - \frac{20}{3}Y\right)R$$
$$\frac{dN_2}{dt} = \left(\frac{4}{3} - \frac{10}{3}Y\right)R \tag{19}$$

where CH_{4dis} and $^{13}CH_{4dis}$ – dissolved combined methane concentration and dissolved concentration of methane which contains the ^{13}C isotopes; CH_{4gas} and $^{13}CH_{4gas}$ –methane content in gaseous phase; B, ^{13C}B – concentrations of methanotrophs that don't contain and contain the ^{13}C isotopes, respectively; ρ_m –maximum specific rate of their consumption; K_{SCH4}, K_{SHNO2} - half saturation constants during the CH_4 and HNO_2 consumption, respectively; Y – the biomass yield coefficient associated with the growth of biomass and reflecting the proportion of substrate transformation into biomass; $\alpha_{CB,}$ α_g – fractionation factors of carbon isotopes during methane oxidation and methane mass-transfer between gaseous and liquid phases, respectively. It was assumed that ammonium was in excess in the studied system. The mass-transfer for carbon dioxide and molecular nitrogen were not considered within the model due to absence of both the experimental data and their effect in the biochemical process. Thus, CO_2, $^{13}CO_2$ and N_2 – concentrations of the corresponding products in the system.

In the reaction (18) the substrates are methane and nitrite and the reaction products are carbon dioxide and nitrogen. The methane is consumed completely, while the nitrite still remains (Pic. 2d). The maximum specific rate of substrate consumption, the biomass yield coefficient and the half- saturation constants are: ρ_m = 0,93 h^{-1}, Y = 0,00185 μM μM^{-1}, K_{SCH4}=2 μM and K_{SHNO2}=43 μM.

When describing the mass transfer process between the liquid and gaseous phases, the equilibrium concentrations of CH_{4dis} and $^{13}CH_{4dis}$ were calculated as $SS=K_H*P_{CH4}$ and $^{13}SS=K_H*P^{13}CH4$, where K_H is Henry's constant, $P_{CH4}= (V_L* CH_{4gas}*22.4/V_G)/1000000$ и $P^{13}CH_4= (V_L* {}^{13}CH_{4gas} *22.4/V_G)/1000000$ are the partial pressures of methane and methane containing ^{13}C, expressed in atm; V_L, V_G - volumes of liquid and gas in the reactor under study in liters, respectively. In model (19), the fractionation factors in the methane oxidation reaction ($\alpha_{CB} = 1.03$) and mass transfer ($\alpha_g = 1.001$) differ significantly according to [Knox et al., 1992]. Thus, the dominant fractionation of carbon isotopes occurs during the microbiological reaction of methane anaerobic oxidation with nitrite but not in the physical process of mass-transfer of gaseous methane into water.

The values of $\delta^{13}C$ signatures for gaseous and dissolved methane, carbon dioxide and biomass were calculated as

$$\delta^{13C}CH_{4\,gas}[‰] = 10^3\left[\frac{^{13C}CH_{4\,gas}/(CH_{4\,gas} - {}^{13C}CH_{4\,gas})}{0.0112372} - 1\right]$$
$$\delta^{13C}CH_{4\,dis}[‰] = 10^3\left[\frac{^{13C}CH_{4\,dis}/(CH_{4\,dis} - {}^{13C}CH_{4\,dis})}{0.0112372} - 1\right] \tag{20}$$

$$\delta^{13C}CO_2[‰] = 10^3\left[\frac{{}^{13C}CO_2/(CO_2 - {}^{13C}CO_2)}{0.0112372} - 1\right]$$

$$\delta^{13C}B[‰] = 10^3\left[\frac{{}^{13C}B/(B - {}^{13C}B)}{0.0112372} - 1\right]$$

Figure 1 shows the Rayleigh graph in double logarithmical coordinates (3), and Figure 2 shows the solution of a dynamic model of the process of methane anaerobic oxidation with nitrite (19). In Fig. 1 and Fig. 2, the same fractionation factor of carbon isotopes ^{12}C и ^{13}C ($\alpha_{CB} = 1.030$) is used. The values in water and gas are quite similar to each other (Fig. 2f, 2i), since $\alpha_g = 1\cancel{,}.01$. If the increase of $\delta^{13C}CO_2$ follows the increase of $\delta^{13C}CH_4$ (Fig. 2 i), the value of ^{13C}B remains approximately constant due to the high concentration of biomass B and a slight increase in it during the reaction (Fig. 2i, 2c). In Fig. 3 the results of a dynamic model with three different factors of carbon isotope fractionation ($\alpha_C = 1.030,\ \alpha_C = 1.025$ and $\alpha_C = 1.035$) are presented. Evidently, $\alpha_C = 1.030$ better fits of the model to the experiment.

Without taking into account the growth of biomass (Y = 0), the stoichiometric equation (18) is simplified to the classical form [Ettwig et al., 2010]:

$$3CH_4 + 8HNO_2 = 3CO_2 + 4N_2 + 10H_2O \qquad (21)$$

We assert that the dynamics of stable isotopes fractionation can follow simple kinetics of the 1st-order in terms of substrate concentration only in exceptional cases. Any mathematical model is based on the inevitable simplification of the properties of a real object. The measurement of isotopic variables and their mathematical description allows, if possible, to look inside the "black box". A description of microbiological processes that take into account isotopes can be quite complicated, since it must always rely on the balance of chemical elements including their isotopes. This becomes justified if new knowledge about the system is revealed at the same time. Thus, in natural ecosystems, a competition among different microorganisms for the same substrate is often observed. Under such conditions, a more complicated dynamic model of the microbial ecosystem including isotopic variables describes the redistribution of the dominance of the substrate consumption processes by various microorganisms and the corresponding change in the fractionation factor of a stable isotope in the substrate [Vavilin, Rytov, 2017; Vavilin et al., 2017].

As outlined in the introduction, the present paper bases on the equations of formal chemical kinetics where the variables are chemical compounds, while mechanism of the biochemical reactions themselves, for example, with the participation of electrons is not considered.

CONCLUSION

Without introducing a specific function of the process rate, the classical static Rayleigh equation for the substrate is derived from the basic dynamic isotropic equation proposed earlier. It is shown that the dynamics of fractionation of stable isotopes follows simple kinetics of the 1st order in terms of substrate concentration only in exceptional cases.

The non-linear model N-DAMO (19) proposed in the article includes a description of the redistribution of the stable carbon isotopes ratio during the reaction. It showed that the dominant fractionation of carbon isotopes occurs during the microbiological reaction of anaerobic oxidation of methane with nitrite, and practically does not depend on the physical process of mass-transfer of gaseous methane into water.

The joint description of the nonlinear dynamics of common and isotopic variables allows us to ~~y~~ analyze mathematically sequential and parallel metabolic pathways of transformations of various substrates in natural and technical chemical-biological systems in one standard means.

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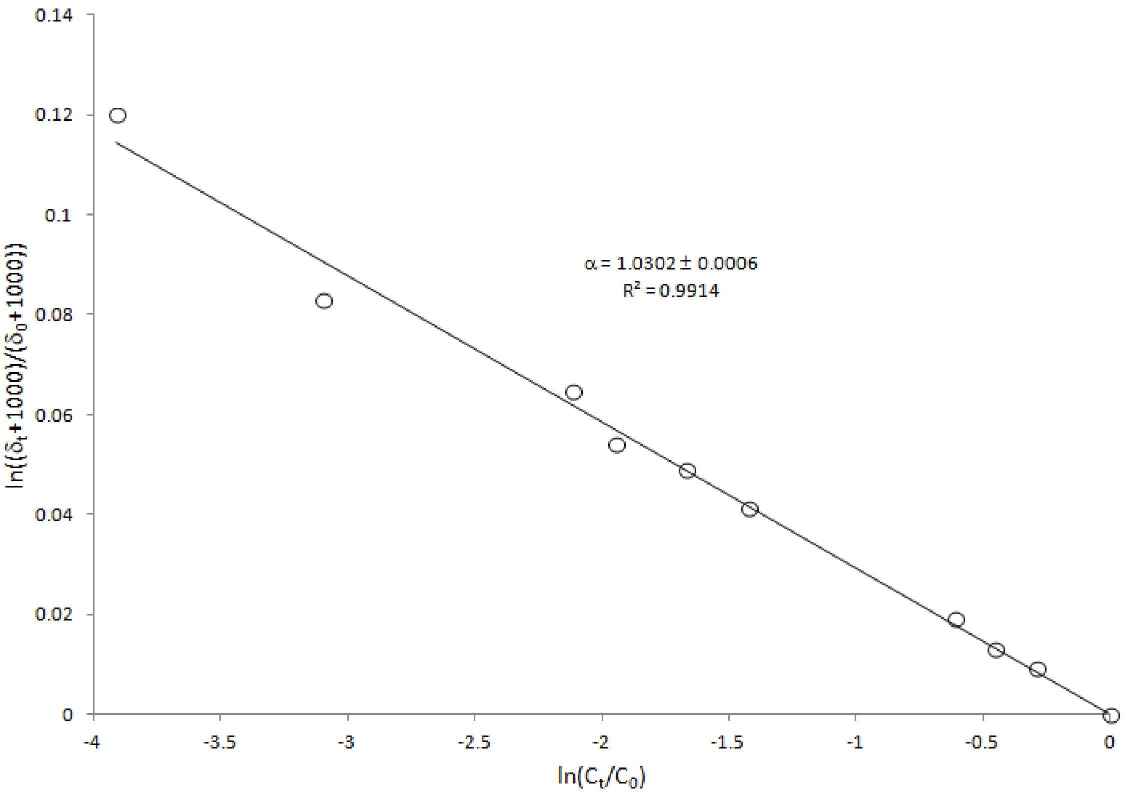

Fig.1. Graphical determination of the carbon isotope fractionation factor by equation (3). Symbols: experiment [Rusigraf et al., 2012].

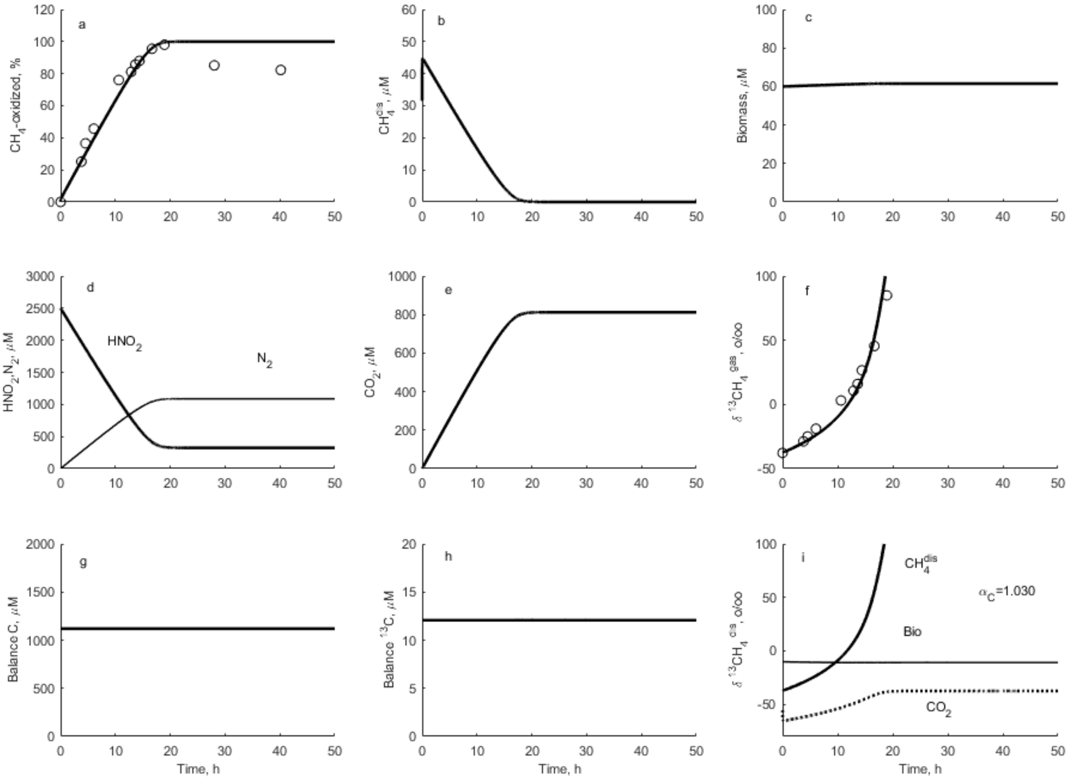

Fig.2. A dynamic model describing the anaerobic oxidation of methane with nitrite at an initial concentration. Symbols: experiment [Rusigraf et al., 2012] of which the last two points at t >25 days were excluded because they were artifacts [Vavilin, Rytov, 2013]; curves: model (19). The ratio of input concentrations of methane and nitrite is 790/2500 =0.316 which is clearly less than 3/8=0.375. Thus, nitrite remains when methane is completely consumed (Fig. 2d).

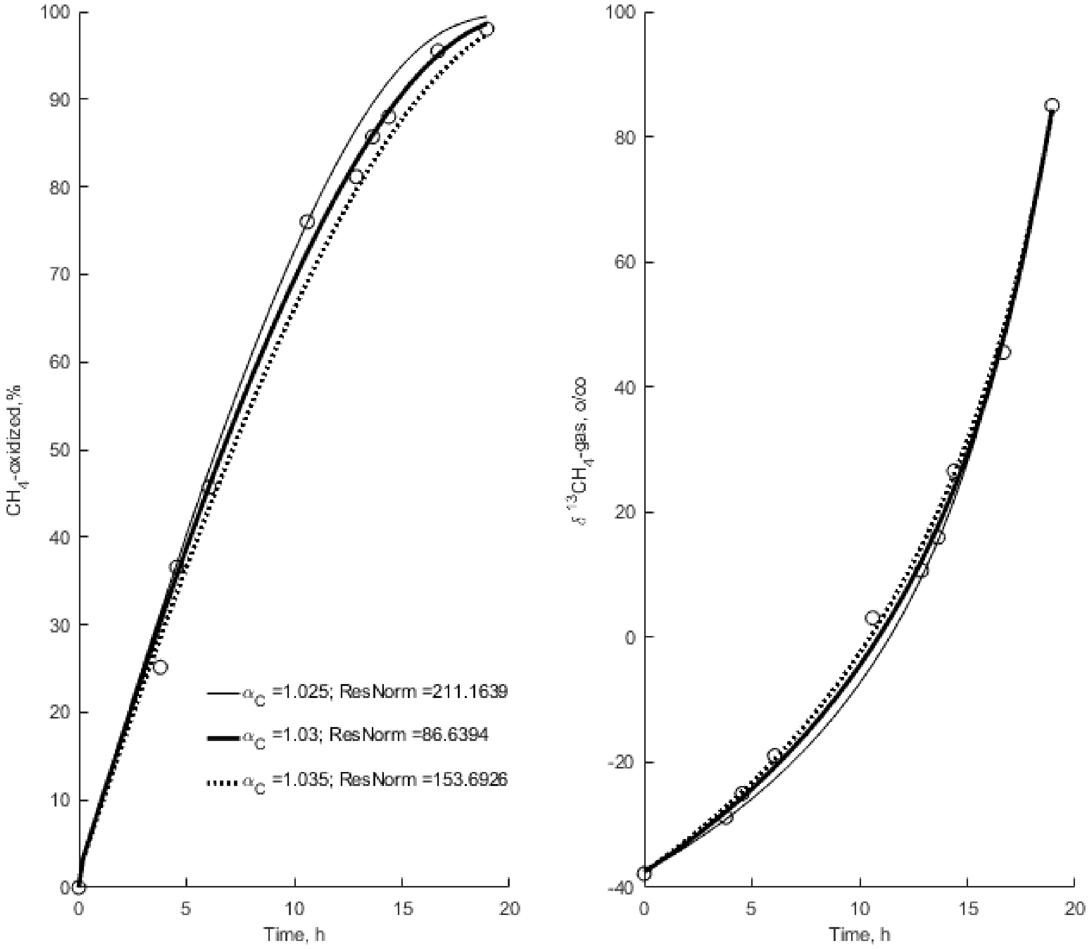

Fig.3. The dynamics of methane oxidation and isotopic signature of methane for three values of fractionation factor of carbon isotope.