**Supplementary Material**

# 1. Isotope fractionation in a flow under steady-state

Considering in a flowing water body the following consecutive chemical reaction:

 (RS1)

Here *k*a and *k*b are rate constants of the two reaction steps. When the concentrations (*c*) of species A, B, and C are under steady-state, the variation of *c*B is 0:

 (S1)

Here *u* is the flow rate; *x* is the distance from the inlet. The solution for *c*B is:

 (S2)

Considering the carbon isotopic fractionation of CH4 during methanogenesis (denoted by “red”) and methanotrophy (denoted by “ox”):

 (RS2)

Using Eq. S2 on reactions in RS2, we have 12CH4, 13CH4, and their ratio of

 (S3)

If 12*k*ox *x/u*→0 (the reaction rate of methanotrophy is slow; the gas sample location is close to the inlet of the water body, or the flow rate is fast), then the approximation of Eq. S3 becomes:

 (S4)

The δ13C formula in Eq. 1 is obtained from Eq. S4 with the definition of δ, 13*α*red = 13*k*red /12*k*red, along with the approximation of ln(1+*x*) = *x* for |*x*| <<1.

If 12*k*ox *x* /*u* >> 1 (the reaction rate of methanotrophy is fast; the gas sample location is far away from the inlet of the water body, and the flow rate is slow), then the approximation of Eq. S3 becomes

 (S5)

Eq. 3 is obtained from Eq. S5 with the definition of δ, kinetic 13C fractionation factor 13*α*red = 13*k*red /12*k*red and 13*α*ox = 13*k*ox /12*k*ox, along with the approximation of ln(1+*x*) = *x* for |*x*| <<1.

Similarly, Eqs. 2 and 4 can be derived based on the following reaction route:

 (RS3)

Here EH and ED are enzyme complexes binding an H and a D atom, respectively. The approximation of ln(1 + *x*) = *x* when |*x*| <<1 is not accurate for δD or *α* less than –200 ‰, but it does not impact the semiquantitative discussion.

For clumped isotopes, considering the steady-state of intermediate species CH in Figure S1:

 (S6)

Subscripts *a*, *b*, *c*, and *d* denote the four consecutive hydrogenation steps during methanogenesis; their following three digits are the number of 13C (0 or 1), the number of D atoms connected to C in the reactant species (valid values are 0, 1, or 2), and the number of D transferred from the hydrogen donor to the carbon-containing species (0 or 1), respectively. Because D/H is about 10–4 <<1, *k*b,001·12CH·D in Eq. S6 can be omitted, then

 (S7)

Similarly,

  (S8)



**Figure S1:** Isotope distribution during methanogenesis and methanotrophy. Isotopologues with more than two substitutions are omitted.

The two digits following subscript “ox” are the number of 13C (valid values are 0 or 1) and the number of D atoms in CH4 (0, 1, or 2), respectively.

For substituted CH4 isotopologues:

 (S9)

Here *α*a,*mij* = *k*a,*mij* / *k*a,000 represents KIE for step *a* in the methanogenesis reaction (similarly for steps *b*, *c* , and *d*); *α*ox,*mi* = *k*ox,*mi* / *k*ox,00 represents KIE in the methanotrophy step.

If the excess clumped isotope effect overwhelms the effect from the difference in the four steps, then for each kind of isotopic substitutions we use the averaged values *α*red,001, *α*red,010, *α*red,011 and *α*red,101 for all the four steps; the expressions of deuterium substituted isotopologues become

 (S10)

Eqs. 5 and 6 are derived from Eqs. S8, S9, and S10, along with the definition of clumped isotope compositions, the definition of excess kinetic clumped isotope fractionation factors 2,13*γ*red = *α*red,101/(*α*red,100 *α*red,001), 2,13*γ*ox = *α*ox,11/(*α*ox,10 *α*ox,01), 2,2*γ*red = *α*red,011/(*α*red,010 *α*red,001), 2,2*γ*ox = *α*ox,02/(*α*ox,012), and ln(1+*x*) = *x* for |*x*| <<1.

If the excess clumped isotope effect is absent, KIE of double isotopic substitutions equals to the product of the two KIEs of the two single isotopic substitutions (Xia and Gao, 2019), then *αa*,011 = *αa*,001 *· αa*,010, *α*ox,02 = *α*ox,01 *· α*ox,01, and so on. It is straightforward from Eqs. S8 and S9 to have

Δ13CH3D = 0 (S11)

The expressions of the 12CH2D2 changes to

(S12)

Then

 (S13)

By reorganizing Eq. S13,

 (S14)

Eqs. S11 and S12 infer that when excess KIE due to clumping is absent, Δ12CH2D2 is negative, which is essentially a combinatorial effect (Yeung, 2016).

# 2. Model details of the Xujiaweizi gas

The forward and backward reactions of H-H and C-H cleavage in H2 alkanes can be described by reaction R7 with *n* = 0, 1, 2, 3; the side reaction (C-C cleavage of alkyls) is described by

C*n*H2*n*+1· + RH → CH3· + C*n*–1H2*n*–1· + R· (RS4)

Here *n* = 2 or 3 represents the cleavage of ethyl and propyl, respectively. This reaction scheme is similar to Almithn and Hibbitts (2019).

Reactions of R7 and RS4 are expanded to cover major isotopologues in the hydrogenolysis of C2H6 and C3H8. The reaction steps and their rate constants are listed in Table S1. Here are necessary simplifications of the model to reduce the complexity and to improve the constraints of the model:

1) Multiple isotopic substitutions (such as 13CH3D) are not considered because of their low abundances.

2) Free radical isomers (such as *n*-propyl and isopropyl) and position-specific isotopic isomers (such as 12CH312CHD· and 12CH2D12CH2·) are not separated; the reaction rates corresponding to the unspecified species (such as propyl and 12CH4D·) can be regarded as an average rate of the reactions separating the isomers.

3) The side reactions (C-C cleavage of alkyls) are not elementary reaction steps; their reaction rates are determined by the C-C cleavage step.

4) Concentration of the free radicals (R·) is assumed to be excessive and constant, so it is omitted in the rate expressions.

5) KIEs are only considered on the cleavage of H-D or C-D bonds in dihydrogen and alkane molecules. These KIEs can be regarded as averaged KIEs of the different steps that an alkane molecule experiences (alkane converting to alkyl, alkyl converting to alkane, and C-C cleavage of alkyl).

The fractional numbers in the reaction rate expressions are derived from symmetry numbers (Xia and Gao, 2019). The fractional numbers in the righthand side of the last two reaction equations in Table S1 are derived from the probabilities of isotopic substitutions and C-C cleavage at different positions.

The initial values (concentrations and isotopic compositions of the species), the rate constants, and the kinetic fractionation factors are listed in Table S2. The initial alkane concentrations are typical values at the onset of the thermal decomposition of C2H6 and C3H8 in sedimentary rocks (Xia and Gao, 2018). The initial isotopic compositions of the alkanes are close to the values of the alkane gas in the Songliao Basin at moderate thermal maturity. The δDH2 value is from the typical value of serpentinization-derived H2 (Sherwood Lollar et al., 2007). The model is not aiming to reconstruct the evolution of the isotopic compositions of the natural gas; instead, it is to demonstrate the isotopic pattern depending on the conversion of C2H6 and C3H8 (expressed as the decrease of wetness). Therefore, the model is dimensionless; the only meaning of the rate constant values is their relative ratios. The overall inverse 13*α* (13*k*/12*k* > 1) and inverse DKIE during the thermal cracking of C2H6 and C3H8 are inferred by a recent study on residual hydrocarbon in organic-rich shale (Xia and Gao, 2018). DKIE of HD cleavage and hydrogen capping is set to unity because the DKIE is overwhelmed by the large δD difference between H2 and organic species.

**Table S1:** Reaction steps and rate constants for hydrogenolysis of C2H6 and C3H8 catalyzed by free radicals.

Reaction Forward reaction rate Backward reaction rate

*Reversible hydrogen extraction:*

H2 + 2R· ↔ 2 RH  *k*0f [H2] *k*0b [RH]2

12CH4 + R· ↔ 12CH3· + RH *k*1f [12CH4] *k*1b [12CH3·] [RH]

12C2H6 + R· ↔ 12C2H5· + RH *k*2f [12C2H6] *k*2b [12C2H5·] [RH]

12C3H8 + R· ↔ 12C3H7· + RH *k*3f [12C3H8] *k*3b [12C3H7·] [RH]

HD + 2R· ↔ RH + RD 1/2 2*α*0 *k*0f [HD] *k*0b [RH][RD]

12CH3D + R· ↔ 12CH2D· + RH 3/4 2*α*1 *k*1f [12CH3D] *k*1b [12CH2D·] [RH]

12CH3D + R· ↔ 12CH3· + RD 1/4 2*α*1 *k*1f [12CH3D]  *k*1b [12CH3·] [RD]

12C2H5D + R· ↔ 12C2H4D· + RH 5/6 2*α*2 *k*2f [12C2H5D] *k*2b [12C2H4D·] [RH]

12C2H5D + R· ↔ 12C2H5· + RD 1/6 2*α*2 *k*2f [12C2H5D]  *k*2b [12C2H5·] [RD]

12C3H7D + R· ↔ 12C3H6D· + RH 7/8 2*α*3 *k*3f [12C3H7D] *k*3b [12C3H6D·] [RH]

12C3H7D + R· ↔ 12C3H7· + RD 1/8 2*α*3 *k*3f [12C3H7D]  *k*3b [12C3H7·] [RD]

13CH4 + R· ↔ 13CH3· + RH 13*α*1 *k*1f [13CH4] *k*1b [13CH3·] [RH]

12C13CH6 + R· ↔ 12C13CH5· + RH 13*α*2 *k*2f [12C13CH6] *k*2b [12C13CH5·] [RH]

12C213CH8 + R· ↔ 12C213CH7· + RH 13*α*3 *k*3f [12C213CH8] *k*3b [12C213CH7·] [RH]

*Irreversible C-C cleavage (side reactions):*

12C2H5· + RH → 2 12CH3· + R· *k*2s [12C2H5·][RH]

12C2H5· + RD → 12CH3· + 12CH2D· + R· *k*2s [12C2H5·][RD]

12C2H4D· + RH → 12CH3· + 12CH2D· + R· *k*2s [12C2H4D·][RH]

12C13CH5· + RH → 12CH3· + 13CH3· + R· *k*2s [12C13CH5·][RH]

12C3H7· + RH → 12CH3· + 12C2H5· + R· *k*3s [12C3H7·][RH]
12C3H7· + RD → 12CH3· + 12C2H4D· + R· *k*3s [12C3H7·][RD]
12C213CH7· + RH → 2/3 12CH3· + 1/3 12C2H5·

 + 1/3 13CH3· + 2/3 12C13CH5· + R· *k*3s [12C213CH7·][RH]

12C3H6D· + RH → 1/2 12CH3· + 1/2 12C2H5·

 + 1/2 12CH2D· + 1/2 12C2H4D· + R· *k*3s [12C3H6D·][RH]

**Table S2:** Parameters for the simulation to resemble Xujiaweizi gas.

 H2 CH4 C2H6 C3H8 R-CH3

Initial concentration 0.5 1 0.03 0.02 1

Initial δ13C (‰) n.a. –30 –28 –27 –15

Initial δD (‰) –700 –200 –180 –160 –80

*k*f *k*0f = 1 *k*1f = 1 *k*2f = 1 *k*3f = 2 n.a.

*k*b *k*0b = 200 *k*1b = 1000 *k*2b = 10 *k*3b = 15 n.a.

*k*s n.a. n.a. *k*2s = 10 *k*3s = 20 n.a.

DKIE 2*α*0 = 1 2*α*1 = 1.04 2*α*2 = 1.02 2*α*3 = 1.02 n.a.

13C KIE n.a. 13*α*1 = 1.006 13*α*2 = 1.005 13*α*3 = 1.005 n.a.

Note: n.a. is not applicable.

# References

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