Physiological acclimation extrapolates the kinetics and thermodynamics of methanogenesis from laboratory experiments to natural environments

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This document describes:

1. Kinetic parameters influenced by physiological acclimation;
2. Kinetic and thermodynamic parameters of *Methanosarcina* and *Methanosaeta*;
3. Reactive transport model;
4. Kinetics of organic carbon degradation;
5. PHREEQC script for reactive transport modeling.

S1. Kinetic parameters influenced by physiological acclimation

The kinetic parameters (i.e., k_a, α_a, and $K_{M,a}$) of the microbes acclimating to substrate concentration $C_{S,a}$ can be related to those of laboratory cultures. To illustrate this point, we substitute equation 10 to 5 and 6, and express the kinetic parameters (i.e., k_o, α_o, and $K_{M,o}$) of laboratory cultures growing at substrate concentration $C_{S,o}$ in terms of the largest-possible affinity constant α_{max} and rate constant k_{max}. The results are

$$\alpha_o = \frac{\alpha_{max}}{1+\sqrt{\frac{\alpha_{max}}{k_{max}}C_{S,o}}}, \tag{A1}$$

and

$$k_o = \frac{\sqrt{\frac{\alpha_{max}}{k_{max}}C_{S,o}}}{1+\sqrt{\frac{\alpha_{max}}{k_{max}}C_{S,o}}}k_{max}. \tag{A2}$$

Combining the two equations,

$$K_{M,o} = \frac{k_o}{\alpha_o} = \sqrt{\frac{k_{max}}{\alpha_{max}}C_{S,o}}. \tag{A3}$$

The kinetic parameters of microbes acclimating to substrate concentration $C_{S,a}$ can also be expressed in terms of α_{max} and k_{max}. The results are

$$\alpha_a = \frac{\alpha_{max}}{1+\sqrt{\frac{\alpha_{max}}{k_{max}}C_{S,a}}}, \tag{A4}$$

and

$$k_{\mathrm{a}} = \frac{\sqrt{\frac{\alpha_{\max}}{k_{\max}} C_{\mathrm{S,a}}}}{1+\sqrt{\frac{\alpha_{\max}}{k_{\max}} C_{\mathrm{S,a}}}} k_{\max} . \tag{A5}$$

Combining the two equations,

$$K_{\mathrm{a}} = \frac{k_{\mathrm{a}}}{\alpha_{\mathrm{a}}} = \sqrt{\frac{k_{\max}}{\alpha_{\max}} C_{\mathrm{S,a}}} . \tag{A6}$$

Comparing equation A3 and A6, we arrive at equation 18 in the main text, i.e.,

$$K_{\mathrm{a}} = K_{\mathrm{o}} \cdot \sqrt{\frac{C_{\mathrm{S,a}}}{C_{\mathrm{S,o}}}} . \tag{A7}$$

From equation A1 and A4,

$$\frac{\alpha_{\mathrm{a}}}{\alpha_{\mathrm{o}}} = \frac{1+\sqrt{\frac{\alpha_{\max}}{k_{\max}} C_{\mathrm{S,o}}}}{1+\sqrt{\frac{\alpha_{\max}}{k_{\max}} C_{\mathrm{S,a}}}} . \tag{A8}$$

Substituting equation A3 into equation A8, we arrive at equation 17 in the main text, i.e.,

$$\alpha_{\mathrm{a}} = \frac{k_{\mathrm{o}}}{K_{\mathrm{M,o}}} \frac{K_{\mathrm{M,o}} + C_{\mathrm{S,o}}}{K_{\mathrm{M,o}} + \sqrt{C_{\mathrm{S,o}} \cdot C_{\mathrm{S,a}}}} . \tag{A9}$$

Finally, we calculate the rate constant k_{a} from equation A7 and A9,

$$k_{\mathrm{a}} = k_{\mathrm{o}} \frac{K_{\mathrm{M,o}} + C_{\mathrm{S,o}}}{C_{\mathrm{S,o}} + K_{\mathrm{M,o}} \cdot \sqrt{\frac{C_{\mathrm{S,o}}}{C_{\mathrm{S,a}}}}} , \tag{A10}$$

which is equation 16 in the main text.

S2. Parameters of acetoclastic methanogens

We compiled the methanogenesis and growth parameters of mesoneutrophilic *Methanosarcina* and *Methanosaeta* laboratory cultures, and the results are shown in table S1. From these results, we calculated specific affinity and ATP yield per methane (table 1). These results confirm the kinetic differences between the two methanogens (Conklin et al., 2006; Min and Zinder, 1989), and provide the foundation for applying the acclimation model.

Table S1. Kinetic and thermodynamic parameters of mesoneutrophilic *Methanosarcina* (*Msr.*) and *Methanosaeta* (*Mse.*) laboratory cultures.

Parameter	Acetate (mM)[a]	pH	T (°C)		Data source
Rate constant of acetate consumption				$mol \cdot g^{-1} \cdot s^{-1}$	
Msr. mazei	50	7.0	37	4.2×10^{-6}	Rajoka et al. (1999)
Mse.soehngenii	50	7.3-7.4	37	4.5×10^{-7}	Huser et al. (1982)
Mse.soehngenii GP6	50	7.0	37	2.7×10^{-7}	Ohtsubo et al. (1992)
Mse.soehngenii FE	50	7.0	37	6.3×10^{-7}	Ohtsubo et al. (1992)
Mse.soehngenii MTAS	50	7.0	37	1.4×10^{-6}	Ohtsubo et al. (1992)
Mse.soehngenii MTKO	50	7.0	37	8.1×10^{-7}	Ohtsubo et al. (1992)
Mse.soehngenii MTHI	50	7.0	37	7.0×10^{-7}	Ohtsubo et al. (1992)
Rate constant of methane production				$mol \cdot g^{-1} \cdot s^{-1}$	
Msr. mazei	50	7.0	37	2.2×10^{-6}	Rajoka et al. (1999)
Msr. barkeri 227	50	6.5-6.7	35	2.4×10^{-6}	Smith and Mah (1980)
	50	n.a.	37	1.7×10^{-6}	Westermann et al. (1989)
Msr. barkeri Fusaro	20	6.8	37	2.7×10^{-6}	Peinemann et al. (1988)
	100	6.9	37	3.3×10^{-7}	Eikmanns and Thauer (1984)
Mse.soehngenii	50	7.3-7.4	37	4.1×10^{-7}	Huser et al. (1982)
Half-saturation constant				mmolal	
Msr. barkeri 227	50	6.5	35-37	5	Smith and Mah (1978)
Msr. barkeri Fusaro	100	6.9	30	3	Schönheit et al. (1982)
	1000	6.3	37	4.1	Wandrey and Aivasidis (1983)
	20	7.0	37	5.7	Fukuzaki et al. (1990)
Mse. concilii	50	7.1-7.5	35	1.2	Patel (1984)
Mse. soehngenii	50	7.0	33	0.46	Zehnder et al. (1980)
Mse. soehngenii	50	7.3-7.4	37	0.7	Huser et al. (1982)
Mse. soehngenii GP6	50	7.0	37	0.84	Ohtsubo et al. (1992)
Mse. soehngenii FE	50	7.0	37	0.39	Ohtsubo et al. (1992)
Mse. soehngenii MTAS	50	7.0	37	0.49	Ohtsubo et al. (1992)
Mse. soehngenii MTKO	50	7.0	37	1.17	Ohtsubo et al. (1992)
Mse. soehngenii MTHI	50	7.0	37	1.19	Ohtsubo et al. (1992)
Biomass yield per acetate				$g \cdot mol^{-1}$	
Msr. acetivorans	50	6.8	35	2.4	Sowers et al. (1984)
Msr. barkeri 227	50	6.8	35	2.7	Sowers et al. (1984)
Msr. barkeri Fusaro	1000	6.3	37	1.5	Wandrey and Aivasidis (1983)
Msr. barkeri MS	50	6.8	35	1.2	Sowers et al. (1984)
	125	5.5-7.5	37	1.1	Hutten et al. (1980)
Msr. mazei	50	7.0	37	6.6	Rajoka et al. (1999)
Mse. concilii	50	7.1-7.5	35	1.15	Patel (1984)
Mse. soehngenii	50	7.3-7.4	37	1.25	Huser et al. (1982)
Biomass yield per methane				$g \cdot mol^{-1}$	
Msr. barkeri 227	50	6.7	35	2.87	Smith and Mah (1980)
	50~100	6.9-7.1	37	2.1	Ferguson and Mah (1983)

	60	7.0	37	2.6	Weimer and Zeikus (1978)
Msr. barkeri MS	60	7.0	37	1.6	Weimer and Zeikus (1978)
Msr. barkeri UBS	60	7.0	37	2.2	Weimer and Zeikus (1978)
Maximum growth rate				s^{-1}	
Msr. acetivorans	50	6.8	35	9.2×10^{-6}	Sowers et al. (1984)
Msr. barkeri 227	50	6.8	35	8.1×10^{-6}	Sowers et al. (1984)
	50	7.0	37-42	3.9×10^{-6}	Maestrojuan and Boone (1991)
Msr. barkeri Fusaro	1000	6.3	37	2.4×10^{-6}	Wandrey and Aivasidis (1983)
	20	7.0	37	1.1×10^{-5}	Fukuzaki et al. (1990)
Msr. barkeri MS	50	7.0	37-42	2.5×10^{-6}	Maestrojuan and Boone (1991)
	50	6.8	35	7.8×10^{-6}	Sowers et al. (1984)
Msr. mazei	50	7.0	37-42	3.6×10^{-6}	Maestrojuan and Boone (1991)
	50	7.0	37-42	2.8×10^{-6}	Maestrojuan and Boone (1991)
Msr. vaculoata	50	7.0	37-42	3.6×10^{-6}	Maestrojuan and Boone (1991)
Mse. soehngenii	50	7.3-7.4	37	1.8×10^{-6}	Huser et al. (1982)
Mse. soehngenii GP6	50	7.1-7.5	35	8.1×10^{-6}	Patel (1984)

(a) Acetate concentration in growth media.

S3. Reactive Transport Model

The reactive transport model accounts for key microbial processes in the profundal sediments of Lake Constance, Germany (fig 5). These processes include:

(1) Acetate production from organic matter by fermenting microbes (AF),
(2) Acetate consumption by aerobic respiration (AR),
(3) Methane consumption by aerobic respiration (MR),
(4) Methane production by *Methanosarcina* (MA1),
(5) Methane production by *Methanosaeta* (MA2), and
(6) The growth of aerobic respirers, *Methanosarcina*, and *Methanosaeta*.

The catabolic reactions of aerobic respirers (AR and MR) are listed in Table S2; the catabolic reactions of the methanogens (MA1 & 2) are given by equation 8. The model consists of the following partial differential equations for acetate (Ac), dioxygen (O_2), dissolved inorganic carbon (DIC), and methane concentrations, and the ordinary differential equations for the biomass concentrations of aerobic respirer (AR), *Methanosarcina* (MA1), and *Methanosaeta* (MA2):

$$\frac{\partial C_{\mathrm{Ac}}}{\partial t} = D_{\mathrm{Ac}} \frac{\partial^2 C_{\mathrm{Ac}}}{\partial x^2} + \frac{1}{\phi}\left(\nu_{\mathrm{Ac,AF}} \cdot r_{\mathrm{AF}} - \nu_{\mathrm{Ac,AR}} \cdot r_{\mathrm{AR}} - \nu_{\mathrm{Ac,MA}} \cdot r_{\mathrm{MA1}} - \nu_{\mathrm{Ac,MA}} \cdot r_{\mathrm{MA2}}\right), \quad \text{(A11)}$$

$$\frac{\partial C_{\mathrm{O_2}}}{\partial t} = D_{\mathrm{O_2}} \frac{\partial^2 C_{\mathrm{O_2}}}{\partial x^2} + \frac{1}{\phi}\left(-\nu_{\mathrm{O_2,AR}} \cdot r_{\mathrm{AR}} - \nu_{\mathrm{O_2,MR}} \cdot r_{\mathrm{MR}}\right), \quad \text{(A12)}$$

$$\frac{\partial C_{\mathrm{DIC}}}{\partial t} = D_{\mathrm{DIC}} \frac{\partial^2 C_{\mathrm{DIC}}}{\partial x^2} + \frac{1}{\phi}\left(\nu_{\mathrm{DIC,AR}} \cdot r_{\mathrm{AR}} + \nu_{\mathrm{DIC,MR}} \cdot r_{\mathrm{MR}} + \nu_{\mathrm{DIC,MA}} \cdot r_{\mathrm{MA1}} + \nu_{\mathrm{DIC,MA}} \cdot r_{\mathrm{MA2}}\right), \quad \text{(A13)}$$

$$\frac{\partial C_{\mathrm{CH_4}}}{\partial t} = D_{\mathrm{CH_4}} \frac{\partial^2 C_{\mathrm{CH_4}}}{\partial x^2} + \frac{1}{\phi}\left(-\nu_{\mathrm{CH_4,MR}} \cdot r_{\mathrm{MR}} + \nu_{\mathrm{CH_4,MA1}} \cdot r_{\mathrm{MA1}} + \nu_{\mathrm{CH_4,MA2}} \cdot r_{\mathrm{MA2}}\right), \tag{A14}$$

$$\frac{dC_{\mathrm{X,AR\&MR}}}{dt} = \left(Y_{\mathrm{X,AR}} \cdot r_{\mathrm{AR}} + Y_{\mathrm{X,MR}} \cdot r_{\mathrm{MR}}\right) \cdot \left(1 - \frac{C_{\mathrm{X,AR\&MR}}}{C_{\mathrm{AR\&MR,max}}}\right) - D \cdot C_{\mathrm{X,AR\&MR}}, \tag{A15}$$

$$\frac{dC_{\mathrm{X,MA1}}}{dt} = Y_{\mathrm{X,MA1}} r_{\mathrm{MA1}} \cdot \left(1 - \frac{C_{\mathrm{X,MA1}} + C_{\mathrm{X,MA2}}}{C_{\mathrm{MA,max}}}\right) - D \cdot C_{\mathrm{X,MA1}}, \tag{A16}$$

and

$$\frac{dC_{\mathrm{X,MA2}}}{dt} = Y_{\mathrm{X,MA2}} r_{\mathrm{MA2}} \cdot \left(1 - \frac{C_{\mathrm{X,MA1}} + C_{\mathrm{X,MA2}}}{C_{\mathrm{MA,max}}}\right) - D \cdot C_{\mathrm{X,MA2}}. \tag{A17}$$

Here $\nu_{\mathrm{Ac,AF}}$ and others are stoichiometric coefficients of chemical species in the catabolic reactions, and r_{AF}, r_{AR}, r_{MR}, r_{MA1}, and r_{MA2} are the rates of AF and other catabolic reactions. We apply the constant concentration and the zero gradient boundary condition at the sediment-water interface and the bottom of the sediment column, respectively.

We calculate the rates of aerobic respiration (i.e., AR and MR) according to the dual-Monod equation. For example, the rate r_{AR} of aerobic respiration coupled to acetate oxidation (AR) is calculated according to

$$r_{\mathrm{AR}} = k_{\mathrm{AR}} \cdot C_{\mathrm{X,AR\&MR}} \cdot \frac{C_{\mathrm{O_2}}}{C_{\mathrm{O_2}} + K_{\mathrm{O_2}}} \cdot \frac{C_{\mathrm{Ac}}}{C_{\mathrm{Ac}} + K_{\mathrm{Ac}}}. \tag{A18}$$

where k_{AR} is the rate constant, $C_{\mathrm{X,AR\&MR}}$ is the biomass concentration, and K_{O2} and K_{Ac} are the half-saturation constants. Here we neglect the thermodynamic control because the energy available from aerobic respiration reaction overwhelms the energy conserved by microbes and the thermodynamic potential factor approaches unity.

Table S2. Rate constant k, half-saturation constants K_{M}, biomass yield Y_{X} per O_2, stoichiometric number χ_{rd} of rate-determining step, and the biomass holding capacity $C_{\mathrm{AR\&MR,max}}$ of aerobic microbes.

Microbial reaction	k [a]	K_{M} (μM)		Y_{X} [a]	χ_{rd} [a]	$C_{\mathrm{AR\&MR,max}}$ [b]
	$\mathrm{mol \cdot g^{-1} \cdot s^{-1}}$	electron donor	O_2 [a]	$\mathrm{mol \cdot g^{-1}}$		$\mu\mathrm{g \cdot cm^{-3}}$
$\frac{1}{2}\mathrm{Acetate} + \mathrm{O_2} = \mathrm{HCO_3^-} + \frac{1}{2}\mathrm{H^+}$	1.0×10^{-6}	50 [a]	0.1	20	8	5.0
$\frac{1}{2}\mathrm{CH_4} + \mathrm{O_2} = \frac{1}{2}\mathrm{HCO_3^-} + \frac{1}{2}\mathrm{H^+} + \frac{1}{2}\mathrm{H_2O}$	1.0×10^{-6}	4.6[c]	0.1	20	8	5.0

Note: (a) Jin and Bethke (2009).

(b) Aerobic microbes in the profundal sediments of Lake Constance has a MPN count of ~5.0×10^6 per ml of sediments (Rothfuss et al., 1997). The maximum biomass concentrations is calculated by assuming the dry weight per cell is 10^{-12} g.

(c) Remsen et al. (1989).

We considered a sediment column of 30 cm long, divided into 30 nodal blocks. We set porosity at 0.9 (Frevert, 1980), and diffusion coefficient at 6×10^{-6} $cm^2\cdot s^{-1}$ (Jin and Bethke, 2009; Kuivila and Murray, 1984). We set very small initial concentrations for chemical compounds and applied an upper boundary condition of pH at 7 (Rahalkar et al., 2009), 2 mM dissolved inorganic carbon (DIC), 50 μM O_2, 3 μM acetate, and 5 μM methane (Frenzel et al., 1990; Stabel, 1986). We assumed that microbes are not motile, and seeded each nodal block with an initial biomass concentration of 1 $\mu g\cdot cm^{-3}$ for aerobic microbes and 10^{-3} $\mu g\cdot cm^{-3}$ for two methanogens. The kinetic and thermodynamic parameters of the two methanogens are computed according to the acclimation model and the parameter values of laboratory cultures (see table 1); the parameters for computing the rates of aerobic respirers are listed in Table S2. We solved for the apparent steady-state distribution of pore-water chemistry and biomass concentrations in the sediment column by running the reactive transport simulation forward from the assumed initial conditions for 500 years, well past the point at which the results stabilized, at about 100 years.

S4. Kinetics of Organic Carbon Degradation

A common model of organic carbon degradation is the first-order one-*G* rate equation (Arndt et al., 2013). According to this equation, the rate r_{AF} of acetate production from organic carbon degradation depends linearly on the concentration C_{OM} of complex insoluble organic carbon,

$$r_{AF} = k \cdot C_{OM}\,, \tag{A19}$$

where k_{AF} is the rate constant in $mol\cdot(g\ TOC)^{-1}\cdot s^{-1}$. In the profundal lake sediments, total organic carbon (TOC) content remained relatively constant in the upper 8 cm of the sediments and decreased steadily with further depth (fig S1A) (Giger et al., 1984; Kappler et al., 2001).

The first-order one *G* model failed in reproducing the concentration profiles of acetate and methane in the sediments at the same time. For example, by combing the first-order rate equation with the TOC depth profile, we obtained a reasonable fit to the methane depth profile by setting the rate constant of organic carbon degradation at 1.6×10^{-12} $mol\cdot g^{-1}\cdot s^{-1}$, but over-predicted the concentrations of acetate (fig S1B and C).

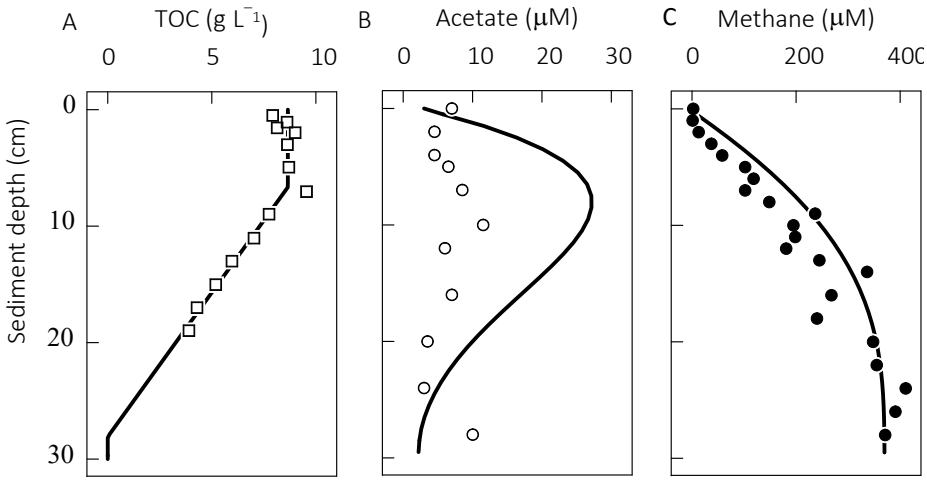

Figure S1. Variations with sediment depth in the concentrations of total organic carbon (TOC in g TOC per L pore fluid, A), acetate (B), and methane (C) in the profundal sediments of Lake Constance, Germany. Data points of TOC (A) are calculated from the TOC content in dry sediment weight reported by Kappler et al. (2001) and by taking the sediment porosity at 85% and the dry weight density at 300 g per L pore fluid (Schulz and Conrad, 1995, 1996); data points of acetate and methane (B and C) are from Schulz and Conrad (1995). Solid line in panel A is the best-fit to the TOC depth profile; lines in panel B and C are the results of reactive transport modeling by using the first-order one-*G* model for organic carbon degradation (eq A19).

S5. PHREEQC script for reactive transport modeling

```
SOLUTION_MASTER_SPECIES
#element/master_species/alkalinity/formula/m.w.
Acetate     HAcetate        0.0     Acetate     59.0
Ox          Ox2             0.0     16.0        32.00
Methane     Methane         0.0     16.0        16.0143

SOLUTION_SPECIES
Methane = Methane
    log_k               0.0
    -delta_h -61.039 kcal

Ox2 = Ox2
    log_k               0.0

HAcetate =  HAcetate
    log_k               0.0

HAcetate =  Acetate- + H+
    log_k               -4.7572

PHASES
FixpH
    H+ = H+
```

```
    log_k 0.0

Ox2(g)
    Ox2 = Ox2
    -log_k   -2.8983
    -analytic -7.5001 7.8981e-3 0.0 0.0 2.0027e5
    -T_c  154.6
    -P_c   49.80
    -Omega 0.021

SOLUTION 0 # Overlying water's element composition
      temp    4
      units    umol/L
      pH       7
      Na       5000      charge
      Cl       5000
      Methane  5
      Acetate    3
      C(+4)    2000
      Ox       50
END

SOLUTION 1-30 # Sedimentary water initially filling nodal blocks
      temp    4
      units    umol/L
      pH       7
      Na       5000      charge
      Cl       5000
      Methane  5
      Acetate    3
      C(+4)    2000
      Ox       5
EQUILIBRIUM_PHASES 1-18
      Fixph     -7.0      NaOH  10.0
END

RATES
AcFM
# Rate of acetate production by fermenting microbes
-start
 10 rate = 5.0e-12  # mol/L/s
 20 SAVE rate * TIME
-end

V_AR
# Rate of aerobic acetate oxidation
-start
 10 rate_s = 0
 20 mD = TOT("Acetate")
 30 mA = TOT("Ox")
 40 rate_con = PARM(1)*0.07  #methanogenesis rate is 7% of the rate obtained in laboratory bioreactors
 50 KD = PARM(2)
 60 KA = PARM(3)
```

```
 70 log_k = PARM(4)
 80 chi = PARM(5)
 90 QoverK = 10^(2*LA("HCO3-") + LA("H+") - LA("Acetate-") - 2*LA("Ox2") - log_k)
100 DG = 8.3143 * TK * LOG(QoverK)
110 ATP_yield = 1
120 aff = QoverK * exp(ATP_yield * 45000 / (8.3143 * TK))
130 tpf = 1.0 - aff^(1/chi)
140 IF (tpf < 0.0) THEN tpf = 0
150 PUT(ATP_yield, 6)
160 rate_s = rate_con * mD * mA /(mD + KD)/(mA + KA) * tpf
170 rate   = rate_s  * KIN("VX_AR")
180 moles  = rate * TIME
190 PUT(rate,   7)
200 SAVE moles
-end
VR_AM
# Rate of aerobic methane oxidation
-start
 10 rate_s = 0
 20 mD = TOT("Methane")
 30 mA = TOT("Ox")
 40 rate_con = PARM(1)*0.07  #methanogenesis rate is 7% of the rate obtained in laboratory bioreactors
 50 KD = PARM(2)
 60 KA = PARM(3)
 70 log_k = PARM(4)
 80 chi = PARM(5)
 90 QoverK = 10^(LA("HCO3-") + LA("H+") + LA("H2O") - LA("Methane") - 2*LA("Ox2") - log_k)
100 DG = 8.3143 * TK * LOG(QoverK)
120 ATP_yield = 1
130 aff = QoverK * exp(ATP_yield * 45000 / (8.3143 * TK))
140 tpf = 1.0 - aff^(1/chi)
150 IF (tpf < 0.0) THEN tpf = 0
160 PUT(ATP_yield, 8)
170 rate_s = rate_con * mD * mA /(mD + KD)/(mA + KA) * tpf
180 rate   = rate_s  * KIN("VX_AR")
190 moles  = rate * TIME
210 PUT(rate,   9)
220 SAVE moles
-end

VX_AR
-start
 10 Xmax = 5
 20 D    = PARM(1)
 30 Y_AR    = GET(6) * 5.0e3
 40 Y_AM   = GET(8)*5.0e3
 50 Xrate = (GET(7) * Y_AR + GET(8)*Y_AM )* (1 - M/Xmax) - D * M
 60 moles = -Xrate * TIME
 70 if (M + moles) < 0 then moles = -M
 80 SAVE moles
-end

VR_M1
```

```
# Rate of methanogenesis by Methanosaeta
# Lab media contain 50e-3 M acetate
-start
 10 rate_s = 0
 20 mD   = TOT("Acetate")
 30 KD = PARM(2) *(50e-3)^(-0.5)*mD^0.5
 40 rate_con = 0.07*PARM(1)*(PARM(2)+50e-3)/(50e-3+sPARM(2)*(50e-3)^0.5*mD^(-0.5))
 50 log_k = PARM(3)
 60 chi  = PARM(4)
 70 FD   = mD/(mD + KD)
 80 QoverK = 10^(LA("HCO3-") + LA("Methane") - LA("H2O") - LA("Acetate-") - log_k)
 90 DG = 8.3143 * TK * LOG(QoverK)
100 ATP_yield = 1.011e-2*(DG/-1000)^1.137
110 IF (ATP_yield > 0.25) THEN ATP_yield = 0.25
120 IF (ATP_yield < 0) THEN ATP_yield = 0
130 aff = QoverK * exp(ATP_yield * 45000 / (8.3143 * TK))
140 tpf = 1.0 - aff^(1/chi)
150 IF (tpf < 0.0) THEN tpf = 0
160 rate_s = rate_con * FD * tpf
170 rate   = rate_s  * KIN("VX_M1")
180 moles  = rate * TIME
190 PUT(ATP_yield, 15)
200 PUT(rate,   16)
210 SAVE moles
-end
VX_M1
-start
 10 Xmax = 0.5
 20 D    = PARM(1)
 30 Y    = GET(15) * 4.35e3
 40 FC   = 1 - (M + KIN("VX_M2"))/Xmax
 50 Xrate = GET(16) * Y * FC - D * M
 60 moles = -Xrate * TIME
 70 if (M + moles) < 0 then moles = -M
 80 SAVE moles
-end

VR_M2
# Rate of methanogenesis by Methanosarcina
-start
 10 rate_s = 0
 20 mD   = TOT("Acetate")
 30 KD = PARM(2) *(50e-3)^(-0.5)*mD^0.5
 40 rate_con = 0.07* PARM(1)*(PARM(2)+50e-3)/(50e-3+PARM(2)*(50e-3)^0.5*mD^(-0.5))
 50 log_k= PARM(3)
 60 chi  = PARM(4)
 70 FD   = mD/(mD + KD)
 80 QoverK = 10^(LA("HCO3-") + LA("Methane") - LA("H2O") - LA("Acetate-") - log_k)
 90 DG = 8.3143 * TK * LOG(QoverK)
100 ATP_yield = 1.011e-2*(DG/-1000)^1.137
110 IF (ATP_yield > 0.5) THEN ATP_yield = 0.5
120 IF (ATP_yield < 0) THEN ATP_yield = 0
130 aff = QoverK * exp(ATP_yield * 45000 / (8.3143 * TK))
```

```
140 tpf = 1.0 - aff^(1/chi)
150 IF (tpf < 0.0) THEN tpf = 0
160 rate_s = rate_con * FD * tpf
170 rate   = rate_s  * KIN("VX_M2")
180 moles  = rate * TIME
190 PUT(ATP_yield, 25)
200 PUT(rate,   26)
210 SAVE moles
-end
VX_M2
-start
 10 Xmax = 0.5
 20 D    = PARM(1)
 30 Y    = GET(25) * 4.35e3
 40 FC   = 1 - (M + KIN("VX_M2"))/Xmax
 50 Xrate = GET(26) * Y * FC - D * M
 60 moles = -Xrate * TIME
 70 if (M + moles) < 0 then moles = -M
 80 SAVE moles
-end

KINETICS 1-30
    AcFM
            -formula Acetate 1.0
    V_AR
            -formula Acetate -1.0 Ox2 -2.0 HCO3 2.0
            -parms   1.0e-9 5.0e-5 1e-6 158.2435 2
            -tol 1e-9
    VR_AM
            -formula Methane -1.0 Ox2 -2.0 HCO3 1.0
            -parms   1.0e-9 4.6e-6 1e-6 155.5420 2
            -tol 1e-9
    VX_AR
            -m0 1
            -formula H 0.0
            -parms   1.0e-8
            -tol 1e-9
    VR_M1
            -formula Acetate -1.0 HCO3 1 Methane 1.0
            -parms   7.1e-10 8e-4 2.7015 2
            -tol 1e-9
    VX_M1
            -m0 0.001
            -formula H 0.0
            -parms   1.0e-8
            -tol 1e-9
    VR_M2
            -formula Acetate -1.0 HCO3 1 Methane 1.0
            -parms   2.3e-9 4.4e-3 2.7015 2
            -tol 1e-9
    VX_M2
            -m0 0.001
            -formula H 0.0
```

```
            -parms   1.0e-8
            -tol 1e-9
TRANSPORT
    -cells            30
    -length           30*0.01         # 1 cm per cell
    -shifts           1
    -time_step    1.58e10         # time, in seconds;
    -flow_direction      diffusion_only
    -boundary_condition   constant closed
    -dispersivity        30*0.0
    -diffusion_coef      6e-10
END
```

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