Supporting Information

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SI Text

Anaerobic Oxidation of Methane as Enzymatically Catalyzed System. The anaerobic oxidation of methane (AOM) is presently understood as two stoichiometrically coupled enzymatic net reaction sequences: methane oxidation, which may essentially represent a reversal of methanogenesis, and dissimilatory sulfate reduction. The involved enzymatic net reactions in a steady state (no change of cellular pools) must take place at the same net rate (Fig. S1, full black arrows) according to the 1:1 stoichiometry CH_4 + $SO_4^{2-} \rightarrow HCO_3^- + HS^- + H_2O$. In contrast, the extent of forward and back fluxes between enzymatic net reactions can differ. If the product is isotopically labeled, the label migrates upstream (arrows and ←) through the intermediate pools. Label concentration in an intermediate decreases with its distance from the product pool. Hence, label back flux through an individual reaction must be greater than the revealed total catabolic back flux.

Time Course Experiment with Consortia from Hydrate Ridge. The results from the time course experiment with highly enriched, detritus-free consortia from Hydrate Ridge are depicted in Fig. S2.

Calculation of the Reverse Reaction Rate. In the catabolic reversible conversion of a substrate to a product, A ⇌ P, the forward and back flux (*Discussion* and Fig. S3) are f_+ and f_- (moles volume^{-1} time^{-1}). When product label, P*, is added, appearance of A* reveals the back flux. If the label is a radioisotope with noticeable decay, error in label quantification is avoided by measuring radioactivity in all samples at the same time after the experiment; the specific isotope decay rate is independent of the chemical composition. The infinitesimal concentration d[A*] formed during an infinitesimal time span dt depends on f_+ and f_-, heavy isotope discrimination in each direction (factors, α_-, $\alpha_+ \geq 1$), and the proportion of label in total product and substrate (brackets indicate concentrations) (Eq. **S1**):

$$\mathrm{d}[\mathrm{A}^*] = f_- \,\mathrm{d}t \frac{1}{\alpha_-}\frac{[\mathrm{P}^*]}{[\mathrm{P}]} - f_+ \mathrm{d}t \frac{1}{\alpha_+}\frac{[\mathrm{A}^*]}{[\mathrm{A}]}. \qquad [\mathbf{S1}]$$

Treatment is simplified by assuming α_-, $\alpha_+ \sim 1$ and that the label proportion in the reactant pool remains very low ([A*]/[A] << [P*]/[P]), and therefore, return by f_+ can be neglected. Eq. **S1** then yields (Eq. **S2**)

$$\mathrm{d}[\mathrm{A}^*] = f_- \,\mathrm{d}t \frac{[\mathrm{P}^*]}{[\mathrm{P}]}. \qquad [\mathbf{S2}]$$

P* is increasingly diluted by steadily forming unlabeled P (Fig. S3). If an increase Δ[A*] is measured after a short enough incubation time, Δt, during which [P*]/[P] remains essentially constant, Eq. **S2** can be simplified and rearranged to (Eq. **S3**)

$$f_- = \frac{\Delta[\mathrm{A}^*]}{\Delta t}\frac{[\mathrm{P}]}{[\mathrm{P}^*]_0} = \frac{\Delta[\mathrm{A}^*]}{\Delta t}\frac{[\mathrm{P}]_0 + [\mathrm{P}^*]_0}{[\mathrm{P}^*]_0}. \qquad [\mathbf{S3}]$$

$[P]_0$ is the initial product concentration, and $[P^*]_0$ is the initial label concentration. If $[P^*]_0 << [P]_0$, Eq. **S3** is further simplified to (Eq. **S4**)

$$f_- = \frac{\Delta[\mathrm{A}^*]}{\Delta t}\frac{[\mathrm{P}]_0}{[\mathrm{P}^*]_0}. \qquad [\mathbf{S4}]$$

Such treatment is similar as the common determination of substrate fluxes in habitats through labeling and measuring product label (1, 2). We applied Eq. **S4**, which corresponds to Eq. **15** (*Materials and Methods*), to evaluate ^{14}C back fluxes in the methane–bicarbonate system.

If, however, label dilution is significant, which it was with the product sulfide, [P] in Eq. **S2** is expressed as a function of time. We assume that the net rate, v, of A → P is largely independent of [A] (zero-order behavior, which is common in many microbial batch incubations) and that cell growth is negligible during incubation. Then, v is constant, and product increases according to $[P] = [P]_0 + [P^*]_0 + vt$ or with $[P^*]_0 << [P]_0$ according to (Eq. **S5**)

$$[\mathrm{P}] = [\mathrm{P}]_0 + vt. \qquad [\mathbf{S5}]$$

The needed variable [A*] (Eq. **S2**) is introduced through $[P^*] = [P^*]_0 - [A^*]$ (mass conservation) leading to (Eq. **S6**)

$$\frac{1}{[\mathrm{P}^*]_0 - [\mathrm{A}^*]}\mathrm{d}[\mathrm{A}^*] = f_- \frac{1}{vt + [\mathrm{P}]_0}\mathrm{d}t. \qquad [\mathbf{S6}]$$

Integration with $[A^*] = 0$ at $t = 0$ yields (Eq. **S7**)

$$\ln\frac{[\mathrm{P}^*]_0}{[\mathrm{P}^*]_0 - [\mathrm{A}^*]} = \frac{f_-}{v}\ln\frac{[\mathrm{P}]_0 + vt}{[\mathrm{P}]_0} \qquad [\mathbf{S7}]$$

or (Eq. **S5**) with resubstitution (Eq. **S8**)

$$\ln\frac{[\mathrm{P}^*]_0}{[\mathrm{P}^*]_0 - [\mathrm{A}^*]} = \frac{f_-}{v}\ln\frac{[\mathrm{P}]}{[\mathrm{P}]_0}. \qquad [\mathbf{S8}]$$

Experimental data displayed in a plot of the left vs. the right argument (Fig. 1*C*, Eq. **16**, and Fig. S2*C*) are, thus, expected to follow a straight-line fit with the slope f_-/v.

In the absence of a net reaction ($v = 0$), direct application of Eqs. **S7** and **S8** is meaningless. Rather, Eq. **S6** must be applied with $v = 0$. Integration yields (Eq. **S9**)

$$\ln\frac{[\mathrm{P}^*]_0}{[\mathrm{P}^*]_0 - [\mathrm{A}^*]} = f_- \frac{t}{[\mathrm{P}]_0} \qquad [\mathbf{S9}]$$

for obtaining f_- ($=f_+$) through a graphic plot, again only as long as label return [A*] → [P*] is negligible. Solving Eq. **S9** for $[A^*] = [A^*](t)$ shows the saturating function, $[A^*] = [P^*]_0 (1 - e^{-(f_-/[P]_0)t})$, again valid as long as label return [A*] → [P*] is insignificant.

For proof of consistency, Eq. **S9** is also derived by transforming the right side of Eq. **S7** according to (Eq. **S10**)

$$\ln\left(1 + \frac{vt}{[\mathrm{P}]_0}\right)^{\frac{f_-}{v}} = \ln\left(1 + \frac{1}{[\mathrm{P}]_0/vt}\right)^{\frac{[\mathrm{P}]_0}{vt}\frac{vt}{[\mathrm{P}]_0}\frac{f_-}{v}} = \ln\left(1 + \frac{1}{[\mathrm{P}]_0/vt}\right)^{\frac{[\mathrm{P}]_0}{vt}\frac{t}{[\mathrm{P}]_0}f_-}. \qquad [\mathbf{S10}]$$

Then, the limit of the argument is formed for $v \rightarrow 0$, which is $[P]_0/(vt) \rightarrow \infty$(Eq. **S11**):

$$\lim_{[\mathrm{P}]_0/(vt)\to\infty}\left(1+\frac{1}{[\mathrm{P}]_0/vt}\right)^{\frac{[\mathrm{P}]_0}{vt}\frac{tf_-}{[\mathrm{P}]_0}}=e^{\frac{tf_-}{[\mathrm{P}]_0}}. \quad \textbf{[S11]}$$

With this equation, Eq. **S7** is written as (Eq. **S12**)

$$\ln\frac{[\mathrm{P}^*]_0}{[\mathrm{P}^*]_0-[\mathrm{A}^*]}=\ln e^{\frac{tf_-}{[\mathrm{P}]_0}}=f_-\frac{t}{[\mathrm{P}]_0}, \quad \textbf{[S12]}$$

which is identical to Eq. **S9**.

Thermodynamics and Kinetics in Catabolism. ***Actual free energy, standard free energy, and equilibrium constant.*** We consider the reaction (**S13**)

$$a\mathrm{A}+b\mathrm{B}\to p\mathrm{P}+q\mathrm{Q}, \quad \textbf{[S13]}$$

where A and B are reactants and P and Q are products. Symbols a, b, p, and q are stoichiometric factors. The free energy of this reaction depends on activities (Eq. **S14**):

$$\Delta G=\Delta G^\circ+RT\ln\frac{\{\mathrm{P}\}^p\{\mathrm{Q}\}^q}{\{\mathrm{A}\}^a\{\mathrm{B}\}^b}. \quad \textbf{[S14]}$$

At equilibrium, $\Delta G = 0$, and therefore (Eq. **S15**),

$$\Delta G^\circ=-RT\ln\left(\frac{\{\mathrm{P}\}^p\{\mathrm{Q}\}^q}{\{\mathrm{A}\}^a\{\mathrm{B}\}^b}\right)_\mathrm{e}=-RT\ln K_\mathrm{e}, \quad \textbf{[S15]}$$

with index e indicating activity at equilibrium. Eq. **S15** can be rewritten as (Eq. **S16**)

$$K_\mathrm{e}=e^{-\Delta G^\circ/(RT)}=10^{-\Delta G^\circ/(RT\ \ln\ 10)}, \quad \textbf{[S16]}$$

with $R = 8.314\ \mathrm{J\ K^{-1}\ mol^{-1}} = 8.314\cdot10^{-3}\ \mathrm{kJ\ K^{-1}\ mol^{-1}}$ and $\ln 10 = 2.303$.

Actual concentrations, equilibrium constant, and actual free energy. For convenience, we now consider a reaction with only one reactant and one product (**S17**):

$$\mathrm{A}\to\mathrm{P}. \quad \textbf{[S17]}$$

The free energy of this reaction is (Eq. **S18**)

$$\Delta G=\Delta G^\circ+RT\ln\frac{\{\mathrm{P}\}}{\{\mathrm{A}\}}, \quad \textbf{[S18]}$$

which can be divided by $-RT$ (Eq. **S19**):

$$\frac{-\Delta G}{RT}=\frac{-\Delta G^\circ}{RT}+\ln\frac{\{\mathrm{A}\}}{\{\mathrm{P}\}}. \quad \textbf{[S19]}$$

Eq. **S19** is brought to an exponential form (Eq. **S20**),

$$e^{-\Delta G/(RT)}=e^{-\Delta G^\circ/(RT)+\ln\{\mathrm{A}\}/\{\mathrm{B}\}}, \quad \textbf{[S20]}$$

which is the same as (Eq. **S21**)

$$e^{-\Delta G/(RT)}=e^{-\Delta G^\circ/(RT)}\frac{\{\mathrm{A}\}}{\{\mathrm{P}\}}. \quad \textbf{[S21]}$$

Using Eq. **S16** introduces K_e in Eq. **S21**, yielding (Eq. **S22**)

$$e^{-\Delta G/(RT)}=K_\mathrm{e}\frac{\{\mathrm{A}\}}{\{\mathrm{P}\}} \quad \textbf{[S22]}$$

or (Eq. **S23**)

$$e^{\Delta G/(RT)}=\frac{\{\mathrm{P}\}}{K_\mathrm{e}\{\mathrm{A}\}}. \quad \textbf{[S23]}$$

With activities ~ concentrations and the connection between the equilibrium constant and the four enzymatic rate constants, $K_\mathrm{e} = k_{+1}\ k_{+2}/(k_{-1}\ k_{-2})$, explained in *SI Text*, *Thermodynamics and Kinetics in Catabolism*, *Kinetic rate constants, equilibrium, and Haldane relationship*, we obtain (Eq. **S24**)

$$e^{\Delta G/(RT)}=\frac{[\mathrm{P}]}{K_\mathrm{e}[\mathrm{A}]}=\frac{k_{-1}k_{-2}[\mathrm{P}]}{k_{+1}k_{+2}[\mathrm{A}]}, \quad \textbf{[S24]}$$

which is used in Eq. 8 in the text (*Discussion*).

Kinetic rate constants, equilibrium, and Haldane relationship. We consider reaction **S17** as being enzymatically catalyzed, with E indicating the enzyme and EA indicating the enzyme–substrate complex (Fig. S4).

Equilibrium conditions (no net reactions and index e indicating equilibrium) are characterized by the equalities (Eq. **S25**)

$$k_{+1}[\mathrm{E}]_\mathrm{e}[\mathrm{A}]_\mathrm{e}=k_{-1}[\mathrm{EA}]_\mathrm{e} \quad \textbf{[S25]}$$

and (Eq. **S26**)

$$k_{-2}[\mathrm{E}]_\mathrm{e}[\mathrm{P}]_\mathrm{e}=k_{+2}[\mathrm{EA}]_\mathrm{e}. \quad \textbf{[S26]}$$

Division of Eq. **S25** by Eq. **S26** eliminates $[\mathrm{E}]_\mathrm{e}$ and $[\mathrm{EA}]_\mathrm{e}$ (Eq. **S27**):

$$\frac{k_{+1}[\mathrm{A}]_\mathrm{e}}{k_{-2}[\mathrm{P}]_\mathrm{e}}=\frac{k_{-1}}{k_{+2}}. \quad \textbf{[S27]}$$

Assuming numerical equality between thermodynamic equilibrium constant (Eqs. **S15** and **S16**) and $[\mathrm{P}]_\mathrm{e}/[\mathrm{A}]_\mathrm{e}$ yields (Eq. **S28**)

$$\frac{k_{+1}k_{+2}}{k_{-1}k_{-2}}=\frac{[\mathrm{P}]_\mathrm{e}}{[\mathrm{A}]_\mathrm{e}}=K_\mathrm{e}. \quad \textbf{[S28]}$$

Strictly speaking, activity coefficients would have to be included. The nominator and denominator are then multiplied with $(k_{-1} + k_{+2})$ (Eq. **S29**):

$$\frac{k_{+1}k_{+2}(k_{-1}+k_{+2})}{k_{-1}k_{-2}(k_{-1}+k_{+2})}=\frac{k_{+2}(k_{-1}+k_{+2})/k_{-2}}{k_{-1}(k_{-1}+k_{+2})/k_{+1}}=\frac{[\mathrm{P}]_\mathrm{e}}{[\mathrm{A}]_\mathrm{e}}. \quad \textbf{[S29]}$$

With $(k_{-1} + k_{+2})/k_{-2} = K_{\mathrm{m}-}$ and $(k_{-1} + k_{+2})/k_{+1} = K_{\mathrm{m}+}$, the Michaelis constants of the back and forward reaction, and $k_{+2} = k_{\mathrm{cat}+}$ and $k_{-1} = k_{\mathrm{cat}-}$ (catalytic constants for product and substrate formation, respectively), Eq. **S29** is written as (Eq. **S30**)

$$\frac{k_{\mathrm{cat}+}K_{\mathrm{m}-}}{k_{\mathrm{cat}-}K_{\mathrm{m}+}}=\frac{k_{\mathrm{cat}+}/K_{\mathrm{m}+}}{k_{\mathrm{cat}-}/K_{\mathrm{m}-}}=\frac{[\mathrm{P}]_\mathrm{e}}{[\mathrm{A}]_\mathrm{e}}, \quad \textbf{[S30]}$$

which is the Haldane relationship. This relationship is usually derived in a different manner by applying the equation for the net rate, v, of a reversible reaction and setting $v = 0$ (3, 4).

Forward and back flux resulting from enzymatic binding and release reactions. We consider a reaction where substrate and product are distinguishable by labeling at the start of the reaction according to Fig. S5.

The enzyme–substrate complex is partly derived from the starting unlabeled substrate designated as $\mathrm{A}^\bullet$ and partly from the starting labeled product designated as P*. Within a very short time span, newly formed A* and $\mathrm{P}^\bullet$ are diluted in the pools of $\mathrm{A}^\bullet$ and P*, respectively, and therefore, return to the side of origin can be neglected. Assuming a steady state with constant concentrations of enzyme–substrate complex, the rate of formation of $\mathrm{EA}^\bullet$ equals the rates of its decomposition. Decomposition to $\mathrm{A}^\bullet$ and $\mathrm{P}^\bullet$ occurs stochastically to both sides, the velocities de-

pending on the proportion of EA• among total enzyme–substrate complex, EA• + EA* (Eq. **S31**):

$$v_{+1} = v_{-1}\frac{[\mathrm{EA}^\bullet]}{[\mathrm{EA}^\bullet]+[\mathrm{EA}^*]} + v_{+2}\frac{[\mathrm{EA}^\bullet]}{[\mathrm{EA}^\bullet]+[\mathrm{EA}^*]}. \quad \textbf{[S31]}$$

Eq. **S31** is rearranged to (Eq. **S32**)

$$\frac{v_{+1}}{v_{-1}+v_{+2}} = \frac{[\mathrm{EA}^\bullet]}{[\mathrm{EA}^\bullet]+[\mathrm{EA}^*]}. \quad \textbf{[S32]}$$

Partition of the flux f_+ in v_{+2} equals partition of EA• in EA• + EA* (Eq. **S33**):

$$\frac{f_+}{v_{+2}} = \frac{[\mathrm{EA}^\bullet]}{[\mathrm{EA}^\bullet]+[\mathrm{EA}^*]}. \quad \textbf{[S33]}$$

Eq. **S33** is expressed with Eq. **S32** as (Eq. **S34**)

$$\frac{f_+}{v_{+2}} = \frac{v_{+1}}{v_{-1}+v_{+2}}. \quad \textbf{[S34]}$$

Because of steady state, $v_{-1} + v_{+2} = v_{+1} + v_{-2}$ (formation of total complex occurs at the same rate as its decomposition), Eq. **S34** can be converted to (Eq. **S35**)

$$f_+ = \frac{v_{+1}v_{+2}}{v_{+1}+v_{-2}}. \quad \textbf{[S35]}$$

An analogous calculation with EP* and f_- yields (Eq. **S36**)

$$f_- = \frac{v_{-1}v_{-2}}{v_{+1}+v_{-2}}. \quad \textbf{[S36]}$$

Forward and back flux expressed by rate constants of an enzyme. The rates in Eq. **S35** can be expressed by the corresponding rate constants (Fig. S4) and concentrations of the respective species. Furthermore, [EA•] + [EA*] = [EA] (total enzyme–substrate complex), and therefore, Eq. **S35** yields (Eq. **S37**)

$$f_+ = \frac{k_{+1}[\mathrm{A}^\bullet]k_{+2}[\mathrm{EA}]}{k_{+1}[\mathrm{A}^\bullet]+k_{-2}[\mathrm{P}^*]}. \quad \textbf{[S37]}$$

In an analogous manner, Eq. **S36** yields (Eq. **S38**)

$$f_- = \frac{k_{-1}[\mathrm{EA}]k_{-2}[\mathrm{P}^*]}{k_{+1}[\mathrm{A}^\bullet]+k_{-2}[\mathrm{P}^*]}. \quad \textbf{[S38]}$$

Because these fluxes also occur (unnoticed) if there is no label, the signs (* and •) used for initial distinction can be omitted. The ratio f_-/f_+ is then written as (Eq. **S39**)

$$\frac{f_-}{f_+} = \frac{k_{-1}k_{-2}[\mathrm{P}]}{k_{+1}k_{+2}[\mathrm{A}]}, \quad \textbf{[S39]}$$

which according to Eq. **S28**, is (Eq. **S40**)

$$\frac{f_-}{f_+} = \frac{[\mathrm{P}]}{K_\mathrm{e}[\mathrm{A}]}. \quad \textbf{[S40]}$$

This equation and Eq. **S24** yield Eq. **9** in the text (*Discussion*). Back flux f_- can be also related to the net rate ($v = f_+ - f_-$) by substituting $f_+ = v + f_-$ (Eq. **S41**):

$$\frac{f_-}{v} = \frac{1}{K_\mathrm{e}[\mathrm{A}]/[\mathrm{P}]-1} \ (\text{for } v \neq 0, [\mathrm{A}]/[\mathrm{P}] \neq 1/K_\mathrm{e}). \quad \textbf{[S41]}$$

To express f_+ and f_- by the concentration of total enzyme, $[\mathrm{E}_0]$ = [E] + [EA], the steady-state equation depicted in Fig. S5 is formulated in the common manner (3) (Eq. **S42**):

$$k_{+1}[\mathrm{E}][\mathrm{A}^\bullet] + k_{-2}[\mathrm{E}][\mathrm{P}^*] = k_{-1}[\mathrm{EA}] + k_{+2}[\mathrm{EA}] \quad \textbf{[S42]}$$

leading to (Eq. **S43**)

$$[\mathrm{E}](k_{+1}[\mathrm{A}^\bullet] + k_{-2}[\mathrm{P}^*]) = (k_{-1}+k_{+2})[\mathrm{EA}]. \quad \textbf{[S43]}$$

Again, omitting label sign included for initial distinction, substituting [E] = $[\mathrm{E}_0]$ − [EA], and solving for [EA] yields (Eq. **S44**)

$$[\mathrm{EA}] = \frac{(k_{+1}[\mathrm{A}]+k_{-2}[\mathrm{P}])[\mathrm{E}_0]}{k_{-1}+k_{+2}+k_{+1}[\mathrm{A}]+k_{-2}[\mathrm{P}]}. \quad \textbf{[S44]}$$

Expressing [EA] in Eq. **S37** by Eq. **S44** yields for the forward flux (Eq. **S45**)

$$f_+ = \frac{k_{+1}[\mathrm{A}]\,k_{+2}[\mathrm{E}_0]}{k_{-1}+k_{+2}+k_{+1}[\mathrm{A}]+k_{-2}[\mathrm{P}]}. \quad \textbf{[S45]}$$

Multiplying nominator and denominator by $(k_{-1} + k_{+2})/k_{+1}k_{-2}$ introduces two composite constants $(k_{-1} + k_{+2})/k_{+1} = K_{\mathrm{m}+}$ and $(k_{-1} + k_{+2})/k_{-2} = K_{\mathrm{m}-}$, the Michaelis constants for the forward and reverse reactions, respectively (Eq. **S46**):

$$f_+ = \frac{K_{\mathrm{m}-}[\mathrm{A}]k_{+2}[\mathrm{E}_0]}{K_{\mathrm{m}+}K_{\mathrm{m}-}+K_{\mathrm{m}-}[\mathrm{A}]+K_{\mathrm{m}+}[\mathrm{P}]}. \quad \textbf{[S46]}$$

Enzyme kinetics often use $k_{+2} = k_{\mathrm{cat}+}$, the catalytic constant (3) for the forward reaction, and $k_{+2}[\mathrm{E}_0] = k_{\mathrm{cat}+}[\mathrm{E}_0] = v_{\mathrm{max}+}$, the maximum forward rate that would be achieved under saturation (that is when totally added enzyme, E_0, is present as enzyme substrate complex and when there is no product causing reverse reaction). Forward flux is thus also expressed as

$$f_+ = \frac{v_{\mathrm{max}+}K_{\mathrm{m}-}[\mathrm{A}]}{K_{\mathrm{m}+}K_{\mathrm{m}-}+K_{\mathrm{m}-}[\mathrm{A}]+K_{\mathrm{m}+}[\mathrm{P}]}. \quad \textbf{[S47]}$$

In an analogous manner we express [EA] in Eq. **S38** by Eq. **S44**, multiply nominator and denominator by $(k_{-1} + k_{+2})/k_{+1}k_{-2}$, and substitute $k_{-1}[\mathrm{E}_0] = k_{\mathrm{cat}-}[\mathrm{E}_0] = v_{\mathrm{max}-}$ (maximum back rate, i.e. with all enzyme present as EA, bound A being product-derived, and free reactant A absent). This leads to the back flux (Eq. **S48**)

$$f_- = \frac{v_{\mathrm{max}-}K_{\mathrm{m}+}[\mathrm{P}]}{K_{\mathrm{m}+}K_{\mathrm{m}-}+K_{\mathrm{m}-}[\mathrm{A}]+K_{\mathrm{m}+}[\mathrm{P}]}. \quad \textbf{[S48]}$$

For proof of consistency, the net rate can be formed by $v = f_+ - f_-$ using Eqs. **S47** and **S48**. This rate yields (Eq. **S49**)

$$v = \frac{v_{\mathrm{max}+}K_{\mathrm{m}-}[\mathrm{A}]-v_{\mathrm{max}-}K_{\mathrm{m}+}[\mathrm{P}]}{K_{\mathrm{m}+}K_{\mathrm{m}-}+K_{\mathrm{m}-}[\mathrm{A}]+K_{\mathrm{m}+}[\mathrm{P}]}, \quad \textbf{[S49]}$$

the classic rate equation of a reversible enzyme reaction with one substrate and one product (3, 4). As long as there is no product (that is [P] = 0) and thus, no back reaction, Eq. **S49** yields (Eq. **S50**)

$$v = \frac{v_{\mathrm{max}+}[\mathrm{A}]}{K_{\mathrm{m}+}+[\mathrm{A}]}, \quad \textbf{[S50]}$$

the well-known rate equation for a unidirectional enzymatic reaction, A → P.

Temperature dependency of reversibility. Below, we present an abbreviated derivation of the van 't Hoff isobar (Vukancic–Vukovic

equation) of the equilibrium constant (full treatment of the topic is in textbooks of physical chemistry).

At constant pressure, the change of the free energy of the reacting system (reactants and products) with temperature is related to the corresponding entropy, as is expressed by (Eq. **S51**):

$$\left(\frac{\partial}{\partial T}\Delta G\right)_p = -\Delta S. \qquad \textbf{[S51]}$$

ΔS is expressed by the free energy definition $\Delta G = \Delta H - T\,\Delta S$ as $\Delta S = (\Delta H - \Delta G)/T$, and therefore (Eq. **S52**),

$$\left(\frac{\partial}{\partial T}\Delta G\right)_p = -\frac{\Delta H - \Delta G}{T} \qquad \textbf{[S52]}$$

or (Eq. **S53**)

$$\left(\frac{\partial}{\partial T}\Delta G\right)_p - \frac{\Delta G}{T} = -\frac{\Delta H}{T}. \qquad \textbf{[S53]}$$

Combining the left terms above (one denominator) and dividing by T yields (Eq. **S54**)

$$\frac{\left(\frac{\partial}{\partial T}\Delta G\right)_p T - \Delta G}{T^2} = -\frac{\Delta H}{T^2}. \qquad \textbf{[S54]}$$

The left side is recognized as the derivative of a quotient if the equation is written as (Eq. **S55**)

$$\frac{\left(\frac{\partial}{\partial T}\Delta G\right)_p T - 1\ \Delta G}{T^2} = \frac{\left(\frac{\partial}{\partial T}\Delta G\right)_p T - \left(\frac{\partial}{\partial T}T\right)\Delta G}{T^2} = -\frac{\Delta H}{T^2}, \qquad \textbf{[S55]}$$

and therefore, the equation is identical with (Eq. **S56**)

$$\left(\frac{\partial}{\partial T}\frac{\Delta G}{T}\right)_p = \frac{-\Delta H}{T^2}. \qquad \textbf{[S56]}$$

This equation allows us (on integration) to calculate the quotient $\Delta G_2/T_2$ at a temperature T_2 from the quotient $\Delta G_1/T_1$ given for another temperature T_1 and hence, also ΔG_2 from a ΔG_1 value. However, there is usually more practical interest in the influence of temperature on the equilibrium constant than on the free energy itself. This preference is accounted for in van 't Hoff's isobar of the equilibrium constant, obtained from Eq. **S56** with $\Delta G^\circ = -RT\ln K_e$, where superscript $^\circ$ indicates standard activities (whereas the temperature can be fixed at any value) (Eq. **S57**):

$$\left(\frac{\partial}{\partial T}\ln K_e\right)_p = \frac{\Delta H^\circ}{RT^2} \qquad \textbf{[S57]}$$

1. Sorokin YI (1962) Experimental investigation of bacterial sulfate reduction in the Black Sea using S^{35}. *Microbiology* 31:329–335.
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3. Bisswanger H (2008) *Enzyme Kinetics: Principles and Methods* (Wiley, New York).
4. Cornish-Bowden A (2004) *Fundamentals of Enzyme Kinetics* (Portland Press, London).

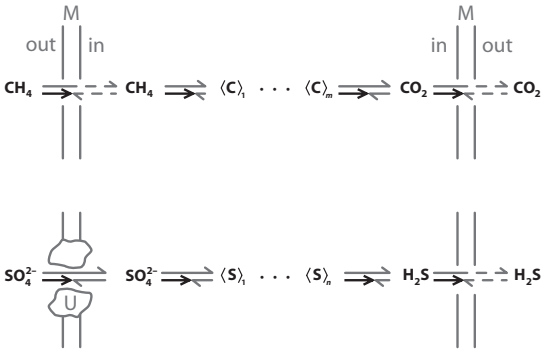

Fig. S1. Schematic illustration of forward flux (⇀), back flux (↽), and net rate (→) during AOM (with *m* and *n* enzymatic steps for methane oxidation and sulfate reduction, respectively); without attribution of organisms and mechanisms. SO_4^{2-} enters the cell through an uptake system (U), whereas CH_4, CO_2, and H_2S are assumed to diffuse rapidly through the cytoplasmic membrane (M) and other layers. ⟨C⟩ and ⟨S⟩ are assumed to be carbon and sulfur intermediates, respectively. Coreactants (such as coenzymes) and coupling between oxidation and reduction (transfer of reducing equivalents) are not depicted. The rates are indicated by the lengths and directions of the arrows (1D vectors). A dynamic steady state (time constant intermediate pools) inside the cell is assumed. In the assumed linear pathways, the net rate is the same for every reaction, whereas the forward and reverse fluxes can differ.

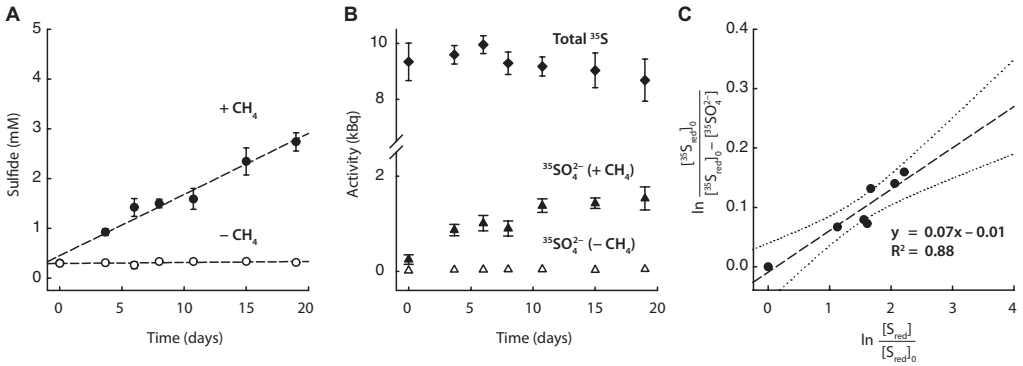

Fig. S2. Time course experiment: addition of labeled sulfide (product) to highly enriched, detritus-free consortia from Hydrate Ridge that perform AOM. (*A*) Developing sulfide concentrations in the presence (●) and absence (○) of methane. (*B*) Development of ^{35}S-activity in the sulfate pool during incubation with (▲) and without (△) methane as electron donor. The total ^{35}S-activity (◆; sulfate + sulfide) is also indicated. The shown radiolabel was determined after incubation at the same time to eliminate the effect of decay. (*C*) Double logarithmic plot according to Eq. **16** to calculate the reverse rate; dotted line marks the 95% confidence interval.

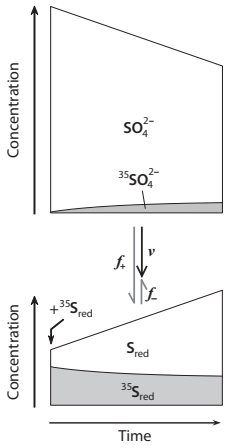

Fig. S3. Schematic illustration of transfer of added sulfide ($^{35}S_{red}$) label (gray) to the sulfate pool by back flux (f_-) during sulfate reduction with constant net rate. The net rate is $v = f_+ - f_-$. Label flux decreases because of label dilution by steadily forming product.

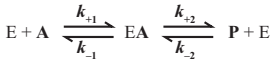

Fig. S4. Simple enzymatic reaction with lumped state (EA) of enzyme-bound substrate.

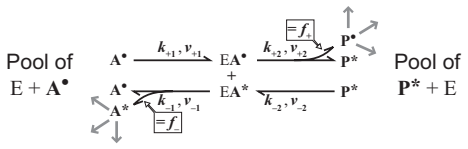

Fig. S5. Simple enzymatic reaction with lumped state (EA) of enzyme-bound substrate. For real or theoretical distinction, the product pool is labeled (P*). The label gradually appears in the substrate pool that was originally unlabeled (A•).

Table S1. Results from incubation experiments with AOM enrichment cultures with ^{14}C and ^{35}S-label

Enrichment culture	AOM rate (μmol mL^{-1} d^{-1}; tracer conversion $^{14}CH_4 \rightarrow {}^{14}CO_2$) + Methane + sulfate	SR rate (μmol mL^{-1} d^{-1}; tracer conversion $^{35}SO_4^{2-} \rightarrow H_2{}^{35}S$) + Methane + sulfate	AOM/SR	MG rate (μmol mL^{-1} d^{-1}; tracer conversion $^{14}CO_2 \rightarrow {}^{14}CH_4$)	
				+ Methane + sulfate	+ Methane − sulfate
Hydrate Ridge	68.0 ± 2.8	70.6 ± 14.5	0.96/1	2.2 ± 0.1	1.6 ± 0.2
Relative to AOM rate (%)	100	103.8		3.2	2.4
Isis Mud Volcano	47.0 ± 6.4	44.0 ± 5.7	1.07/1	2.6 ± 0.2	1.1 ± 0.3
Relative to AOM rate (%)	100	93.7		5.5	2.3

Results are illustrated in Fig. 2. AOM, anaerobic oxidation of methane; MG, methanogenesis; SR, sulfate reduction.

Table S2. Net sulfate reduction rate and tracer-based calculated reverse reaction during incubation of AOM enrichment cultures

Enrichment culture	SR rate (μmol mL^{-1} d^{-1}; quantified chemically)		Reverse SR rate (% of SR; tracer conversion $H_2{}^{35}S \rightarrow {}^{35}SO_4^{2-}$)*	
	+ Methane	− Methane	+ Methane	− Methane
Hydrate Ridge	0.12	0.002		
Relative to SR rate (%)	100	1.6	7 ± 1	nd
Isis Mud Volcano	0.19	0.009		
Relative to SR rate (%)	100	4.7	13 ± 1	nd

Rates shown in Tables S1 and S2 are different, because Table S1 was an experiment using the entire biomass (short time, only starting and endpoint measurements), whereas Table S2 was a long-time batch experiment in a time series (*Materials and Methods*). Results are illustrated in Fig. 2, and derivation of the data is depicted in Fig. 1 and Fig. S2. SR, sulfate reduction.
*Directly determined with Eq. 16.

Table S3. Description of terms, fluxes, rates, labels, and pools

Symbol	Description	Units
A	Substrate	$mol \cdot L^{-1}$
P	Product	$mol \cdot L^{-1}$
$A^\bullet$	Unlabeled substrate	$mol \cdot L^{-1}$
P*	Labeled product	$mol \cdot L^{-1}$
A*	Substrate derived by back flux from labeled product (P*)	$mol \cdot L^{-1}$
$P^\bullet$	Unlabeled product derived by forward flux from unlabeled substrate ($A^\bullet$)	$mol \cdot L^{-1}$
E	Enzyme	$mol \cdot L^{-1}$
EA	Lumped enzyme-bound state	
v	Experimentally accessible net rate for a process, such as a single-step reaction or a multistep enzymatically catalyzed process	$mol \cdot L^{-1} \cdot s^{-1}$
n	Number of steps in a process; for the case of a simple enzymatic reaction including a lumped enzyme-bound state, $n = 2$	
$v_{+1}, v_{+2}, \ldots, v_{+n}$	Individual forward rates in a process consisting of n steps	$mol \cdot L^{-1} \cdot s^{-1}$
$v_{-1}, v_{-2}, \ldots, v_{-n}$	Individual back rates in a process consisting of n steps	$mol \cdot L^{-1} \cdot s^{-1}$
$v^\bullet_{+1}, v^\bullet_{+2}, \ldots, v^\bullet_{+n}$	Individual forward rates for substrate label ($^\bullet$) in a process consisting of n steps	$mol \cdot L^{-1} \cdot s^{-1}$
$v^*_{+1}, v^*_{+2}, \ldots, v^*_{+n}$	Individual forward rates for product label (*) in a process consisting of n steps; because dilution of product label (*) in substrate pool is large at an initial stage of a labeling experiment, $v^*_{+1} \sim 0$	$mol \cdot L^{-1} \cdot s^{-1}$
$v^*_{-1}, v^*_{-2}, \ldots, v^*_{-n}$	Individual back rates for product label (*) in a process consisting of n steps	$mol \cdot L^{-1} \cdot s^{-1}$
$v^\bullet_{-1}, v^\bullet_{-2}, \ldots, v^\bullet_{-n}$	Individual back rates for unlabeled substrate ($^\bullet$) in a process consisting of n steps; because dilution of unlabeled product ($^\bullet$) in product pool is large at an initial stage of a labeling experiment, $v^\bullet_{-n} \sim 0$	$mol \cdot L^{-1} \cdot s^{-1}$
f_+	Corresponds to $v^\bullet_{+n}$: experimentally accessible flux of substrate arriving in product pool (increase in $P^\bullet$)	$mol \cdot L^{-1} \cdot s^{-1}$
f_-	Corresponds to v^*_{-1}: experimentally accessible flux of substrate arriving in substrate pool (increase in A*)	$mol \cdot L^{-1} \cdot s^{-1}$
k_{+1}	Rate constant in enzymatically catalyzed reaction for conversion of substrate (A) to enzyme-bound state (EA)	$(mol \cdot L^{-1})^{-1} \cdot s^{-1}$
k_{-1}	Rate constant in enzymatically catalyzed reaction for conversion of enzyme-bound state (EA) to substrate (A)	s^{-1}
k_{+2}	Rate constant in enzymatically catalyzed reaction for conversion of enzyme-bound state (EA) to product (P)	s^{-1}
k_{-2}	Rate constant in enzymatically catalyzed reaction for conversion of product (P) to enzyme-bound state (EA)	$(mol \cdot L^{-1})^{-1} \cdot s^{-1}$