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THE THERMODYNAMICS AND KINETICS OF MICROBIAL METABOLISM

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ABSTRACT. The various kinetic rate laws commonly used to describe microbial metabolism are derived considering only forward reaction progress and hence are inconsistent with the requirements of thermodynamics. These laws may be applied without significant error where abundant energy is available to drive the metabolic reaction, so the forward reaction overwhelms the reverse. The laws are, however, unsuitable where little energy may be available. In previous papers we derived a new rate law for microbial respiration considering that reaction progresses simultaneously in both the forward and reverse directions. In this paper, we demonstrate in a new and rigorous way how the rate law can account quantitatively for the thermodynamic driving force for reaction. We refine our previous work on microbial respiration to better account for details of the electron transfer process. We furthermore extend the theory to account for enzymatic reaction and microbial fermentation. We show that commonly used rate laws of simple form can be modified to honor thermodynamic consistency by including a thermodynamic potential factor. Finally, we consider how the rate of biomass synthesis can be determined from the rate of respiration or fermentation. We apply these results to describe (1) the enzymatic reaction by which benzoyl-CoA forms, (2) crotonate fermentation, and (3) glucose fermentation; for each process we demonstrate how the reaction rate is affected by the thermodynamic driving force. Results of the study improve our ability to predict microbial metabolic rates accurately over a spectrum of geochemical environments, including under eutrophic and oligotrophic conditions.

INTRODUCTION

A microorganism liberates chemical energy from its environment by using its enzymes to catalyze a chemical reaction. A respiring microbe catalyzes a redox reaction in which electrons are transferred from a donor to acceptor species, leaving the donor oxidized and the acceptor reduced. It conserves a portion of the energy liberated in this way by translocating protons outside of the cell's membrane and synthesizing adenosine triphosphate (ATP) from adenosine diphosphate (ADP) and the orthophosphate ion (PO_4^{3-}, denoted P_i). A fermenting microbe, in contrast, conserves energy by coupling ATP synthesis to the breakdown of a complex substrate molecule found in its environment. The breakdown produces simpler species, one more oxidized and the other more reduced than the substrate. The ATP serves as a store of chemical energy in a respiring or fermenting microbe, which it can expend for purposes such as cell maintenance and reproduction, biomass synthesis, and chemical species transport across its membrane.

A microorganism, then, affects the chemistry of its environment by catalyzing chemical reactions. The environment, on the other hand, controls the activities of

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microorganisms by providing habitats, nutrients, and energy resources and in this way the community structures of microbial populations. Predicting accurately the rate of microbial metabolism in nature is fundamental to understanding interaction between microbial populations and their geochemical environments.

The rate laws commonly used to predict microbial respiration rate, such as the Monod and dual-Monod equations, make no accounting of the energetics of the metabolic process. They do not consider, for example, the requirement that the environment supply at least as much energy as is conserved by microbial metabolism. The relations are developed assuming that the respiration reaction proceed in the forward direction only, a reasonable assumption only where the environment is rich in chemical energy, that is where the metabolic reaction is far from equilibrium.

Chemical reactions in natural environments in many cases can supply only modest amounts of energy to a microbe because they deviate little from thermodynamic equilibrium. This is especially likely to be true for anaerobic respiration and the fermentation of short-chain fatty acids like propionate and butyrate. In such cases, we must acknowledge the metabolic reaction proceeds simultaneously in the forward and reverse directions; the net rate is the difference between the forward and reverse rates. Predicting the rate of microbial metabolism in natural environments in such cases requires use of a kinetic rate law consistent with the principles of thermodynamics, one that accounts for the reverse as well as forward progress of the metabolic reaction.

The need for kinetic rate laws in general to be thermodynamically consistent is broadly appreciated among physical chemists (for example, Denbigh, 1961; Blum and Luus, 1964; Van Rysselberghe, 1967; Boudart, 1975, 1976; Boyd, 1977; Corio, 1983; Boudart and Djega-Mariadassou, 1984; Peka and Miloslav, 2005), following pioneering work on the subject done in the 1940s and 1950s (Gadsby and others, 1946; Horiuti, 1948; Manes and others, 1950; Hollingsworth, 1952a, 1952b, 1957). Microbiologists have been able to avoid thermodynamic considerations in their kinetic calculations in large part because they tend to consider situations where large amounts of energy are available to drive a metabolic reaction forward. Most laboratory experiments, for example, are formulated to facilitate rapid microbial growth, and therefore contain abundant quantities of substrates. Similarly, some polluted environments, such as where organic compounds are exposed to atmospheric dioxygen, are energetically rich. In such cases, the energy available in the environment is sufficiently in excess of the energy conserved by the microbe such that the forward reaction rate overwhelms the reverse rate, allowing the latter to be ignored.

The Earth's hydrosphere and lithosphere, including many pristine and contaminated environments, however, are not everywhere rich in chemical energy. The amount of energy released by organic matter degradation, for example, may be small due to the nature of the reaction, or the fact that the substrate is depleted. In these cases, reverse reaction may not be negligible, and we cannot necessarily ignore the requirement that a cell conserve by its metabolic reaction part of the energy available from environment.

In studying metabolic rates in many natural environments, therefore, geomicrobiologists need to employ a thermodynamically consistent rate law. Failure to do so presents clear contradictions. The Monod equation (Monod, 1949), for example, predicts that a microorganism will continue to metabolize its substrate until its concentration asymptotically approaches zero. But as its concentration decreases, the energy a substrate can offer to microbes diminishes, and eventually metabolic reactions may become energetically unfavorable. In behaving as suggested by the Monod equation, a microbe might need to expend energy to drive forward its own metabolic reaction.

In recent papers (Jin and Bethke, 2002, 2003), we derived on the basis of the chemiosmotic model of respiration and from nonequilibrium thermodynamics a general rate law for microbial respiration accounting for the thermodynamic control on respiration rate. In this paper, we show in a new and rigorous way how the rate law for microbial metabolism in general can take into account the thermodynamic driving force, the difference between the energy available and the energy conserved. We extend the rate law to account for the kinetics of enzymatic reactions and the rate of microbial fermentation. We then show that rate laws in common use, such as the Monod equation, can be modified to be thermodynamically consistent. We take enzymatic reaction of benzoyl-CoA formation as an example and show that thermodynamic driving force controls the progress of enzymatic reactions. Finally, using microbial fermentation of crotonate and glucose as examples, we demonstrate how thermodynamic driving forces control the rates of microbial fermentation. These examples provide concrete illustrations of why honoring thermodynamic consistency can be important when predicting the rates of enzymatic reaction and microbial metabolism in natural environments.

THERMODYNAMIC CONSISTENCY

There exists a natural consistency between the fields of chemical kinetics and thermodynamics that must be honored by any general theory of reaction rates (for example, Boudart, 1976). The equilibrium state of a chemical reaction, for example, is the state at which forward and reverse reaction rates are in balance. These concepts are familiar in geochemistry. The rate r (mol $\cdot$ sec^{-1}) at which a mineral dissolves or precipitates (in latter case, r is negative), for example, represents the difference between the rate of mineral dissolution (forward reaction) and that of mineral precipitation (reverse reaction) and can be predicted by a rate law of a form

$$r = k_+ A_S \prod_i [A_i]^{\alpha_i} \left[1 - \left(\frac{Q}{K} \right)^{1/\chi} \right] \tag{1}$$

(Lasaga, 1981, 1984; Aagaard and Helgeson, 1982; Nagy and others, 1991; Nagy and Lasaga, 1992). Here, k_+ is the rate constant for the forward reaction, A_S is the mineral's surface area, $[A_i]$ is the concentration of a chemical species A_i, and α_i is the exponent associated with this species, Q and K are the reaction's ion activity product and equilibrium constant, and, as will be discussed later, χ is the average stoichiometric number. This equation can be re-expressed

$$r = k_+ A_S \prod_i [A_i]^{\alpha_i} \left[1 - \exp\left(-\frac{f}{\chi RT} \right) \right] \tag{2}$$

in terms of the thermodynamic driving force f, which is the negative of the Gibbs free energy change ΔG of reaction (that is $f = -\Delta G$); here, R is the gas constant and T is absolute temperature. Where the reaction is in equilibrium, dissolution and precipitation are in balance. At the equilibrium point, f is zero and, by this equation, the net reaction rate vanishes.

In this section, we consider in a rigorous sense the requirement of consistency between kinetics and thermodynamics. We begin by taking the overall reaction between an initial reactant species Sp_1 and a final product species Sp_N

$$Sp_1 \rightleftarrows Sp_N \tag{3}$$

Reaction proceeds simultaneously in both the forward and reverse directions, at rates of r_+ and r_-, respectively. The Gibbs free energy change of the reaction is taken as ΔG,

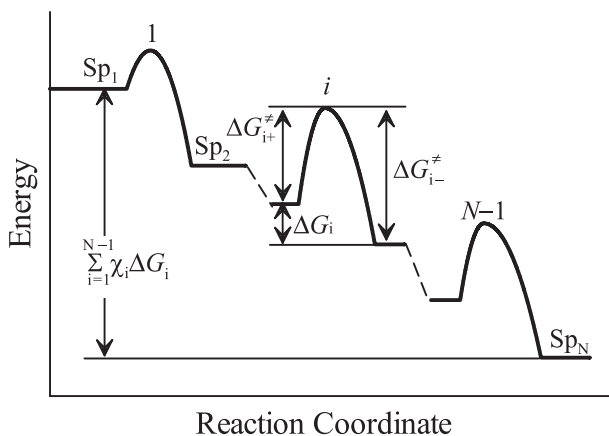Fig. 1. Variation with reaction progress of chemical energy for the overall reaction 3 (Jin and Bethke, 2002). The reaction is composed of $N-1$ elementary steps. The Gibbs free energy change ΔG_i for an elementary step i is the difference $\Delta G_{i+}^{\neq} - \Delta G_{i-}^{\neq}$ between the activation energies for the step's forward and reverse reactions.

the negative of which is the thermodynamic driving force f. As shown in figure 1, the overall reaction is composed of $N-1$ elementary steps,

$$\mathrm{Sp}_1 \overset{1}{\rightleftarrows} \mathrm{Sp}_2 \cdots \mathrm{Sp}_i \overset{1}{\rightleftarrows} \mathrm{Sp}_{i+1} \cdots \mathrm{Sp}_{N-1} \overset{N-1}{\rightleftarrows} \mathrm{Sp}_N \qquad (4)$$

Here, Sp_i ($i = 2, \ldots, N-1$) is a reaction intermediate. Each of the elementary steps i occurs χ_i times per turnover of reaction 3, where χ_i is the step's stoichiometric number. The Gibbs free energy change and thermodynamic driving force for step i are taken as ΔG_i and f_i ($f_i = -\Delta G_i$). The Gibbs free energy change for the overall reaction is

$$\Delta G = \sum_i \chi_i \Delta G_i \qquad (5)$$

since step i occurs χ_i times per turnover of the overall reaction 3. The thermodynamic driving force for the overall reaction then can be written

$$f = \sum_i \chi_i f_i \qquad (6)$$

in terms of the driving force f_i for step i.

Elementary Reaction

According to the activated complex theory (Lasaga, 1981), the forward and reverse rates (r_{i+} and r_{i-}) for an elementary step i (between Sp_i and Sp_{i+1}) are given as

$$r_{i+} = \frac{k_B T}{\hbar} \exp\left(-\frac{\Delta G_{i+}^{\neq}}{RT}\right) \qquad (7)$$

and

$$r_{i-} = \frac{k_B T}{\hbar} \exp\left(-\frac{\Delta G_{i-}^{\neq}}{RT}\right) \qquad (8)$$

Here, k_B is Boltzmann's constant, $\hbar$ is Plank's constant, and $\Delta G_{i+}^{\neq}$ and $\Delta G_{i-}^{\neq}$ are the Gibbs free energy of activation for forward and reverse reaction at step i. The Gibbs free energy released over step i, ΔG_i, is the difference $\Delta G_{i+}^{\neq} - \Delta G_{i-}^{\neq}$ between the activation energies, as can be seen in figure 1. Combining equations (7) and (8),

$$\frac{r_{i+}}{r_{i-}} = \exp\left(-\frac{\Delta G_{i+}^{\neq} - \Delta G_{i-}^{\neq}}{RT}\right) = \exp\left(-\frac{\Delta G_i}{RT}\right) = \exp\left(\frac{f_i}{RT}\right) \tag{9}$$

we see the ratio of forward to reverse rate for the step varies with the thermodynamic driving force. The step's net rate r_i is the difference between r_{i+} and r_{i-} and can be written as

$$r_i = r_{i+}\left[1 - \exp\left(\frac{\Delta G_i}{RT}\right)\right] \tag{10}$$

or

$$r_i = r_{i+}\left[1 - \exp\left(-\frac{f_i}{RT}\right)\right] \tag{11}$$

by substituting equation (9).

Overall Reaction

The ratio of the forward to reverse rates of the overall reaction can be expressed in terms of those for individual elementary steps (see Appendix)

$$\frac{r_+}{r_-} = \prod_{i=1}^{N-1} \frac{r_{i+}}{r_{i-}} \tag{12}$$

Substituting equation (9), the ratio for the overall reaction is given

$$\frac{r_+}{r_-} = \exp\left(-\sum_{i=1}^{N-1} \Delta G_i \middle/ RT\right) = \exp\left(\sum_{i=1}^{N-1} f_i \middle/ RT\right) \tag{13}$$

This expression can be rewritten

$$\frac{r_+}{r_-} = \exp\left(-\frac{\Delta G}{\chi RT}\right) = \exp\left(\frac{f}{\chi RT}\right) \tag{14}$$

where χ is the average stoichiometric number, defined as

$$\chi = \frac{\sum_{i=1}^{N-1} \chi_i \Delta G_i}{\sum_{i=1}^{N-1} \Delta G_i} = \frac{\sum_{i=1}^{N-1} \chi_i f_i}{\sum_{i=1}^{N-1} f_i} \tag{15}$$

Substituting equation (5) and (6)

$$\chi = \frac{\Delta G}{\sum_{i=1}^{N-1} \Delta G_i} = \frac{f}{\sum_{i=1}^{N-1} f_i} \tag{16}$$

(Temkin, 1963).

The net rate r of overall reaction is the difference between the forward and reverse rates, that is $r = r_+ - r_-$. Substituting equation (14) into this relation gives

$$r = r_+ \cdot F_T \tag{17}$$

where F_T is the thermodynamic potential factor

$$F_T = 1 - \exp\left(\frac{\Delta G}{\chi RT}\right) = 1 - \exp\left(-\frac{f}{\chi RT}\right) \tag{18}$$

(Happel, 1972). This factor shows how the overall reaction rate depends on the thermodynamic driving force f for the reaction. Where there is a strong drive, ΔG is large and negative, F_T approximates unity, and the overall rate is about equal to the forward reaction rate. If the reaction is in equilibrium, the driving force f and hence F_T vanishes, and the net rate is zero. Where f is negative, F_T takes a negative value and the reaction proceeds backwards, that is, at a negative rate.

Equations (17) and (18) are notable in that they show that a rate law, in order to be thermodynamically consistent, needs to account for not only the forward but the reverse rate of reaction. The net rate, the difference between these two values, varies with the amount of energy available to drive the reaction, and this variation is accounted for by the thermodynamic potential factor F_T. These relations constitute important tenets of irreversible thermodynamics and must be included in any general theory of reaction rate.

MICROBIAL METABOLISM

Applying the discussion in the previous section, we can show the rate of microbial metabolism can be calculated in a way that honors the requirement of thermodynamic consistency.

Enzymatic Reactions

The basic unit of microbial metabolism is the enzymatic reaction, that is, a reaction catalyzed by an enzymatic protein. To catalyze a reaction, an enzyme binds first to the reactant or substrate compound, S. Once bound, the substrate-enzyme complex reacts to form the product species P. An enzymatic reaction can be represented in a general form as

$$\sum_S \nu_S S \rightleftarrows \sum_P \nu_P P \tag{19}$$

where ν_S and ν_P are the stoichiometric coefficients for the substrate and product species. The Gibbs free energy change (ΔG) of the reaction is

$$\Delta G = \Delta G^o + RT \ln \frac{\Pi_P \, a_P^{\nu_P}}{\Pi_S \, a_S^{\nu_S}} \tag{20}$$

where ΔG^o is the Gibbs free energy change under standard conditions: absolute temperature of T (in Kelvin) and the activities of reactant and products (that is, a_S and a_P, respectively) and gas fugacities of 1. In cases of dilute solutions and gases, following common practice in geomicrobiology, we will use concentration and partial pressure in place of activity and fugacity when evaluating this equation.

A special case of enzymatic reaction occurs where the Gibbs free energy change becomes positive (that is $\Delta G > 0$), making forward progress of the reaction thermodynamically unfavorable. Enzymes can still catalyze such a reaction by simultaneously catalyzing an energy-releasing reaction, such as the hydrolysis of ATP to adenosine monophosphate (AMP) and pyrophosphate (PP$_i$), or to ADP and P$_i$. In the case of ATP hydrolysis to AMP and PP$_i$
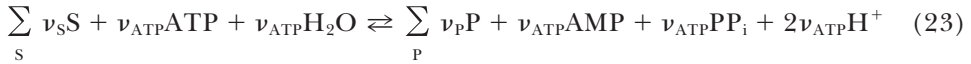
$$ATP + H_2O \rightleftarrows AMP + PP_i + 2H^+ \tag{21}$$
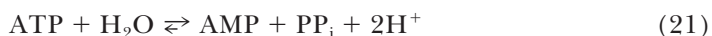
the Gibbs free energy change ΔG_M is

$$\Delta G_M = \Delta G_M^{o'} + RT \ln \frac{[AMP][PP_i]}{[ATP]} \tag{22}$$

where [ ] represents concentration of chemical species (in molal unit) and $\Delta G_M^{o'}$ is the value of ΔG_M under biological standard conditions (that is activities and fugacities of 1 and pH 7), about -41.67 kJ $\cdot$ (mol ATP)$^{-1}$ at 25°C (Thauer and others, 1977).

If a number ν_{ATP} of ATPs are hydrolyzed per turnover of reaction 19, the amount of energy released is the product of this number and ΔG_M, that is $\nu_{ATP} \cdot \Delta G_M$. The overall reaction takes the general form

$$\sum_S \nu_S S + \nu_{ATP} ATP + \nu_{ATP} H_2O \rightleftarrows \sum_P \nu_P P + \nu_{ATP} AMP + \nu_{ATP} PP_i + 2\nu_{ATP} H^+ \tag{23}$$

and the thermodynamic driving force f for the reaction

$$f = -\Delta G - \nu_{ATP} \cdot \Delta G_M \tag{24}$$

is the difference between the energy available from reaction 19 (that is the negative of ΔG, eq 20) and that from the hydrolysis of ATP to AMP and PP_i. Typical examples of coupled enzymatic reactions are substrate uptake into a cell's cytoplasm (for example, Varma and others, 1983; Stahlmann and others, 1991) and substrate activation reactions, such as acetyl-CoA formation (Cozzone, 1998) and adenosine phosphosulfate formation (Peck, 1959).

Fermentation

Fermentative microorganisms can conserve the chemical energy released from the oxidation of organic compounds without transferring electrons to an external electron acceptor. Here external means the electron acceptor is not produced or consumed by the fermentation reaction. During fermentation, organic compounds are degraded and oxidized to a series of intermediate compounds. The electrons released from the oxidation are utilized to take coenzymes, such as nicotinamide adenine dinucleotide (NAD$^+$), to their reduced form. The reduced coenzymes revert to their oxidized form by reducing intermediate compounds. As a result, microbial fermentation degrades an organic substrate S to reaction products of two forms, one oxidized (P$^+$) and the other reduced (P$^-$)

$$S \rightleftarrows \sum_{P^+} \nu_{P^+} P^+ + \sum_{P^-} \nu_{P^-} P^- \tag{25}$$

The energy available ΔG_A for fermentation is the negative of the Gibbs free energy change ΔG of this reaction, that is

$$\Delta G_A = -\Delta G = -\Delta G^o - RT \ln \frac{\Pi_{P^+} [P^+]^{\nu_{P^+}} \Pi_{P^-} [P^-]^{\nu_{P^-}}}{[S]} \tag{26}$$

Fermentative microorganisms can conserve a fraction of the energy released by coupling reaction 25 to ATP synthesis

$$ADP + P_i + 2H^+ \rightleftarrows ATP + H_2O \tag{27}$$

This pathway is known as substrate level phosphorylation. The Gibbs free energy change ΔG_P of ATP synthesis is

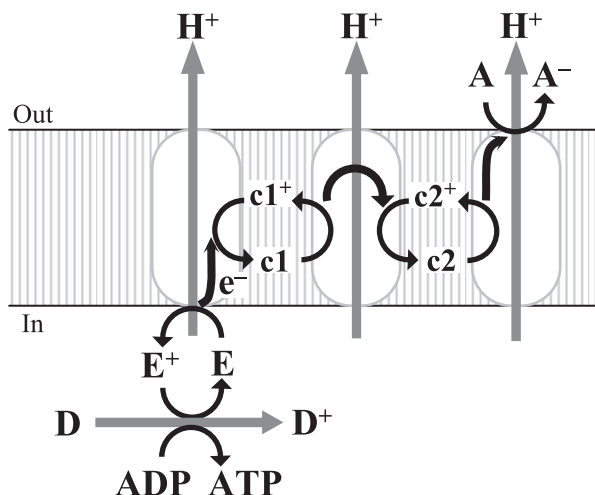$$\Delta G_P = \Delta G_P^{o'} + RT \ln \frac{[ATP]}{[ADP][P_i]} \tag{28}$$

Fig. 2. Generalized model of microbial respiration. Electrons derived from the oxidation of an electron donating species D are transferred to redox coenzyme E. The energy released in this step is conserved by synthesizing ATP from ADP and phosphate. The electrons released from the oxidation of the reduced coenzyme E are then transferred through the respiratory chain containing coenzymes c1 and c2 to an accepting species A. Some of the energy released is conserved by translocating protons out of cell membrane, building up proton motive force. Reaction centers (ovals) are, from left to right: primary reductase, coenzyme reductase, and a terminal reductase.

where $\Delta G_P^{o'}$, the standard Gibbs free energy change at pH 7, is about 31.50 kJ · (mol ATP)$^{-1}$ at 25°C (Rosing and Slater, 1972), [ATP], [ADP], and [P$_i$] are concentrations of ATP, ADP, and phosphate inside the cell membrane. The value of ΔG_P, denoted as the phosphorylation potential, ranges from 40 to 50 kJ · (mol ATP)$^{-1}$ in anaerobic microorganisms (Thauer and others, 1977; Kashket, 1983; Bond and Russell, 1998; Tran and Unden, 1998).

The overall reaction for microbial fermentation, then, is

$$S + \nu_P ADP + \nu_P P_i + 2\nu_P H^+ \rightleftarrows \sum_{P^+} \nu_{P^+} P^+ + \sum_{P^-} \nu_{P^-} P^- + \nu_P ATP + \nu_P H_2O \quad (29)$$
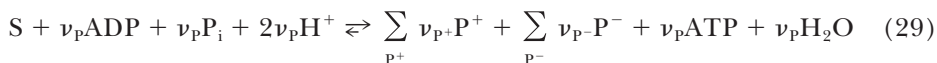
where ν_P is the number of ATP synthesized per turnover of the reaction. The total amount of energy ΔG_C conserved during fermentation then is the product $\nu_P \cdot \Delta G_P$. Note the value of ΔG_P and thus ΔG_C is positive. The thermodynamic driving force f for fermentation

$$f = \Delta G_A - \Delta G_C$$
$$= \Delta G_A - \nu_P \cdot \Delta G_P \quad (30)$$

is the difference between the energy available (eq 26) and that conserved. This value represents the chemical energy available to drive the degradation of substrate S while synthesizing ATP.
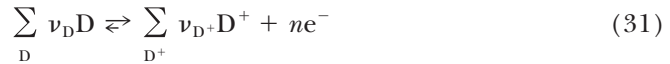
Respiration

Respiring microorganisms derive energy by catalyzing an electron transfer reaction. Electrons derived from the oxidation of an electron donor pass through a respiratory chain to a terminal enzyme, when they reduce an external electron acceptor (fig. 2). Among the broad variety of electron donors found in natural environments are dihydrogen (H$_2$), formate, and acetate. Common electron acceptors

are dioxygen (O_2), nitrate, ferric iron, sulfate, bicarbonate, and so on. The respiratory chain itself is a series of membrane-associated enzymes and coenzymes, which conserve a part of the chemical energy released from the electron transfer reaction as proton motive force across the cell membrane, an energy store which the cell can use to synthesize ATP.

   Redox reaction.—Many electron donors such as acetate and propionate cannot donate electrons directly to the respiratory chain. Instead, electrons pass into the respiratory chain through the cycling of a redox coenzyme E. Oxidation of an electron donor D

$$\sum_{D} \nu_D D \rightleftharpoons \sum_{D^+} \nu_{D^+} D^+ + ne^- \tag{31}$$

to D^+ is coupled to the reduction of a redox coenzyme E^+

$$\sum_{E^+} \nu_{E^+} E^+ + ne^- \rightleftharpoons \sum_{E} \nu_E E \tag{32}$$

to form E . It is coenzyme E that donates electrons to the respiratory chain. As electrons pass through the cell's respiratory chain to electron accepting species, they shuttle along a series of redox enzymes and coenzymes. A primary redox enzyme, the first enzyme in the chain, strips electrons from the coenzyme E according to

$$\sum_{E} \nu_E E \rightleftharpoons \sum_{E^+} \nu_{E^+} E^+ + ne^- \tag{33}$$
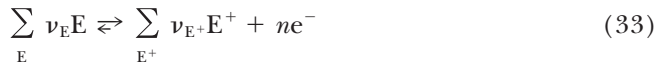
Once electrons have traversed the respiratory chain, a terminal redox enzyme transfers them to an accepting species, according to a half-cell reaction

$$\sum_{A} \nu_A A + ne^- \rightleftharpoons \sum_{A^-} \nu_{A^-} A^- \tag{34}$$

where A and A^- are chemical species on the oxidized and reduced sides of the electron accepting reaction.

   We can write the electron transfer from the electron donors D to acceptors A as two consecutive redox reactions. The first is the oxidation of donor D (reaction 31) and reduction of coenzyme E^+ (reaction 32),

$$\sum_{D} \nu_D D + \sum_{E^+} \nu_{E^+} E^+ \rightleftharpoons \sum_{D^+} \nu_{D^+} D^+ + \sum_{E} \nu_E E \tag{35}$$
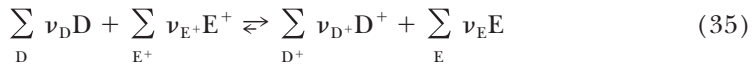
The second the re-oxidation of redox coenzyme E (reaction 33) to pass electrons into the respiratory chain and eventually onto the electron acceptor A (reaction 34),

$$\sum_{E} \nu_E E + \sum_{A} \nu_A A \rightleftharpoons \sum_{E^+} \nu_{E^+} E^+ + \sum_{A^-} \nu_{A^-} A^- \tag{36}$$
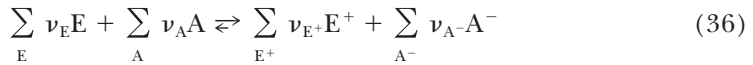
The redox reaction occurring in the environment as a result of microbial respiration is
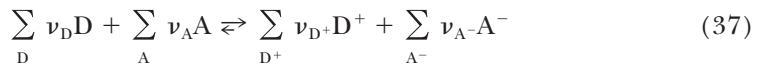
$$\sum_{D} \nu_D D + \sum_{A} \nu_A A \rightleftharpoons \sum_{D^+} \nu_{D^+} D^+ + \sum_{A^-} \nu_{A^-} A^- \tag{37}$$
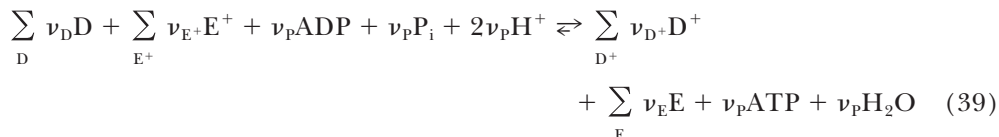
the sum of reaction 35 and 36. The energy available for respiration can be calculated as the negative value of the Gibbs free energy change of reaction

$$\Delta G_A = -\Delta G = nF\Delta E^\circ - RT \ln \frac{\Pi_{D^+} [D^+]^{\nu_{D^+}} \Pi_{A^-} [A^-]^{\nu_{A^-}}}{\Pi_D [D]^{\nu_D} \Pi_A [A]^{\nu_A}} \tag{38}$$

where F is the Faraday's constant and ΔE^o is the redox potential difference under standard conditions between half-reactions 34 and 31.

Energy conservation.—Respiring microorganisms can conserve some of the energy released by electron transfer from donor to acceptor in two ways. First, if the metabolism involves a redox coenzyme E, the microbe can couple the reduction of E during electron acceptance (reaction 35) to ATP synthesis (reaction 27). The overall reaction is

$$\sum_{D} \nu_D D + \sum_{E^+} \nu_{E^+} E^+ + \nu_P ADP + \nu_P P_i + 2\nu_P H^+ \rightleftarrows \sum_{D^+} \nu_{D^+} D^+$$
$$+ \sum_{E} \nu_E E + \nu_P ATP + \nu_P H_2O \quad (39)$$

the sum of the two reactions. The amount of energy conserved is the product $\nu_P \cdot \Delta G_P$ of the number ν_P of ATPs synthesized and the phosphorylation potential.

Second, according to the chemiosmotic theory (Mitchell, 1961), microorganisms can conserve the chemical energy released from the electron transfer through the respiratory chain (reaction 36) by translocating protons inside their cytoplasm to the outside of their membrane

$$H_{in}^+ \rightleftarrows H_{out}^+ \quad (40)$$

Here H_{in}^+ and H_{out}^+ represent, respectively, protons inside and outside the membrane (fig. 2). We can represent the coupled reaction between electron transfer and proton translocation in a general form

$$\sum_{E} \nu_E E + \sum_{A} \nu_A A + \nu_H^R H_{in}^+ \rightleftarrows \sum_{E^+} \nu_{E^+} E^+ + \sum_{A^-} \nu_{A^-} A^- + \nu_H^R H_{out}^+ \quad (41)$$
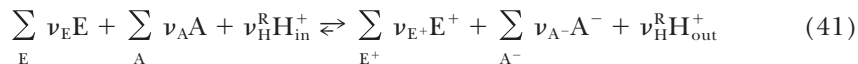
where ν_H^R is the number of protons translocated outside the membrane per turnover of the reaction.

Translocating protons outside the cell leads to differences in electrical charge and proton concentration across the cell membrane, creating electrical potential and chemical potential differences. When protons pass outside the membrane, the movement of a charge of $+1$ against the electrical potential difference across the membrane leads to an electrical energy change, the amount of which is given per mole of protons as $F\Delta\psi$. Here, $\Delta\psi = \psi_{out} - \psi_{in}$ is the difference between the electrical potential outside (ψ_{out}) and inside (ψ_{in}) the membrane. The movement of protons through the concentration difference across the membrane results in a chemical energy change, the amount of which per mole of protons is $RT \ln [H_{out}^+]/[H_{in}^+]$. The sum of the two energy changes

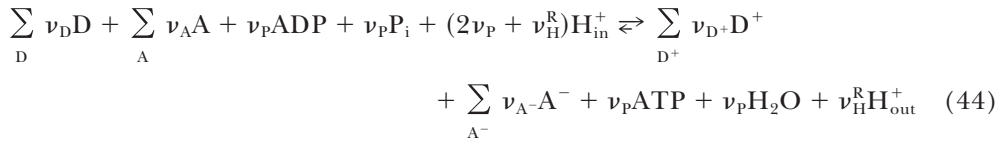$$\Delta G_H = F\Delta\psi + RT \ln\frac{[H_{out}^+]}{[H_{in}^+]} = F\Delta p \quad (42)$$

represents the total free energy change in $kJ \cdot (mol\ H^+)^{-1}$ arising from the translocation of protons outside the cell membrane. Here, Δp is the proton motive force, in volts (Nicholls and Ferguson, 1992)

$$\Delta p = \Delta\psi - \frac{RT \ln(10)}{F} \Delta pH \quad (43)$$

and $\Delta pH = pH_{out} - pH_{in}$, the difference between the pH outside (pH_{out}) and inside (pH_{in}) the membrane. The total amount of energy conserved by translocating the

number of $\nu_{\mathrm{H}}^{\mathrm{R}}$ protons is the product $\nu_{\mathrm{H}}^{\mathrm{R}} \cdot F \cdot \Delta p$ of the number of protons translocated and the free energy change ΔG_{H}.

The overall reaction representing microbial respiration can be written

$$\sum_{\mathrm{D}} \nu_{\mathrm{D}} \mathrm{D} + \sum_{\mathrm{A}} \nu_{\mathrm{A}} \mathrm{A} + \nu_{\mathrm{P}} \mathrm{ADP} + \nu_{\mathrm{P}} \mathrm{P_i} + (2\nu_{\mathrm{P}} + \nu_{\mathrm{H}}^{\mathrm{R}}) \mathrm{H_{in}^+} \rightleftarrows \sum_{\mathrm{D^+}} \nu_{\mathrm{D^+}} \mathrm{D^+}$$

$$+ \sum_{\mathrm{A^-}} \nu_{\mathrm{A^-}} \mathrm{A^-} + \nu_{\mathrm{P}} \mathrm{ATP} + \nu_{\mathrm{P}} \mathrm{H_2O} + \nu_{\mathrm{H}}^{\mathrm{R}} \mathrm{H_{out}^+} \quad (44)$$
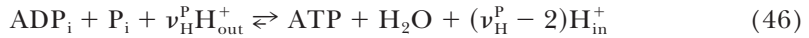
as the sum of reactions 39 and 41. The total amount of energy conserved (ΔG_{C}) during electron transfer from the electron donor D to electron acceptor A is

$$\Delta G_{\mathrm{C}} = \nu_{\mathrm{P}} \cdot \Delta G_{\mathrm{P}} + \nu_{\mathrm{H}}^{\mathrm{R}} \cdot F \cdot \Delta p \quad (45)$$

the sum of the energy conserved as phosphorylation potential during reaction 39 and that conserved as proton motive force during reaction 41.

The phosphorylation potential ΔG_{P} and proton motive force Δp in respiring microorganisms are interconvertible due to the catalytic activities of ATP synthase in the cell membrane. This enzyme can transfer proton motive force to the phosphorylation potential, and vice versa, by coupling ATP synthesis to proton translocation from the outside to inside of the cell membrane,

$$\mathrm{ADP_i} + \mathrm{P_i} + \nu_{\mathrm{H}}^{\mathrm{P}} \mathrm{H_{out}^+} \rightleftarrows \mathrm{ATP} + \mathrm{H_2O} + (\nu_{\mathrm{H}}^{\mathrm{P}} - 2) \mathrm{H_{in}^+} \quad (46)$$

Here, $\nu_{\mathrm{H}}^{\mathrm{P}}$ is the number of protons translocated out of cell membrane per ATP synthesized. The Gibbs free energy change of this reaction is

$$\Delta G = \Delta G_{\mathrm{P}} - \nu_{\mathrm{H}}^{\mathrm{P}} \cdot F \cdot \Delta p \quad (47)$$

In contrast to microbial respiration (reaction 44), reaction 46 can be assumed to be close to thermodynamic equilibrium. As a result, the Gibbs free energy change ΔG is zero and

$$\Delta G_{\mathrm{P}} = \nu_{\mathrm{H}}^{\mathrm{P}} \cdot F \cdot \Delta p \quad (48)$$

Substituting into equation (45), we can express the total amount ΔG_{C} of energy conserved

$$\Delta G_{\mathrm{C}} = \nu_{\mathrm{P}}^{\mathrm{R'}} \cdot \Delta G_{\mathrm{P}} \quad (49)$$

in terms of phosphorylation potential. Here $\nu_{\mathrm{P}}^{\mathrm{R'}} = \nu_{\mathrm{P}} + \nu_{\mathrm{H}}^{\mathrm{R}} / \nu_{\mathrm{H}}^{\mathrm{P}}$ is the number of equivalent ATPs synthesized during respiration.

The thermodynamic driving force f for microbial respiration

$$f = \Delta G_{\mathrm{A}} - \nu_{\mathrm{P}}^{\mathrm{R'}} \cdot \Delta G_{\mathrm{P}} \quad (50)$$

is the difference between the energy available (ΔG_{A}, eq 38) and that conserved (ΔG_{C}, eq 49). The value of f represents the chemical energy available to drive forward the transfer of electrons from donor D through the respiratory chain and acceptor A, while conserving energy by ATP synthesis and proton translocation.

Biomass Synthesis

Among the uses for the energy a microorganism conserves during fermentation or respiration is the creation of new biomass. The ability to predict the rate at which a strain creates biomass is important to understanding biogeochemical reactions, because the reactions are autocatalytic, that is, the reaction rates attainable in a geochemical system increase as biomass concentration increases.

Biomass synthesis requires that nutrients, primarily carbon and nitrogen, be consumed. Organic as well as inorganic carbon can be utilized as the source of nutrient carbon, so we can take the general formula CH_pO_q to represent the carbon source (Stouthamer and Van Verseveld, 1985). The reductance degree γ_C of the carbon source is the number of electrons available per carbon atom, given by

$$\gamma_C = 4 + p - 2q \tag{51}$$

as shown by Minkevich and Eroshin (1973). If we neglect the phosphorus and sulfur content of the biomass formed, we can write a general formula $C_5H_7O_2N$ to represent biomass (Hoover and Porges, 1952). The reductance degree γ_B for biomass is

$$\gamma_B = 4 + \frac{7}{5} - 2 \cdot \frac{2}{5} - 3 \cdot \frac{1}{5}$$
$$= 4 \tag{52}$$

The difference z between γ_B and γ_C (that is $z = \gamma_B - \gamma_C = 2q - p$) represents the number of electrons consumed ($z > 0$) or liberated ($z < 0$) per carbon during biomass synthesis.

For certain carbon nutrients such as propionate ($CH_3CH_2COO^-$) and butyrate ($CH_3CH_2CH_2COO^-$), the reduction degree γ_C of the carbon source is greater than that of the biomass, γ_B, in which case the value of z becomes negative and hence biomass synthesis releases electrons. Where bicarbonate (HCO_3^-) and formate ($HCOO^-$) are utilized as the carbon source, however, the value of z is greater than zero and we can represent biomass synthesis as an electron accepting half-reaction

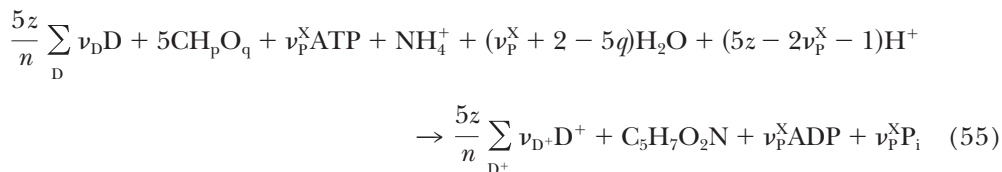$$5CH_pO_q + NH_4^+ + (5z - 1)H^+ + 5ze^- \rightarrow C_5H_7O_2N + (5q - 2)H_2O \tag{53}$$

assuming the microorganism utilizes ammonia as its nitrogen source. During biomass synthesis, the electrons consumed are provided by the oxidation of the redox coenzymes, and the energy required is provided by the hydrolysis of ATP (the reverse of reaction 27). The overall reaction representing biomass synthesis, then,

$$\frac{5z}{n} \sum_E \nu_E E + 5CH_pO_q + \nu_P^S ATP + NH_4^+ + (5z - 2\nu_P^S - 1)H^+ \rightarrow \frac{5z}{n} \sum_{E^+} \nu_{E^+} E^+$$
$$+ C_5H_7O_2N + \nu_P^S ADP + \nu_P^S P_i + (5q - 2 - \nu_P^S)H_2O \tag{54}$$
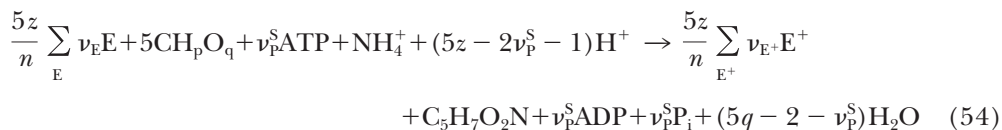
is the sum of half-cell reactions 33 and 53. Here ν_P^S is the mole number of ATPs hydrolyzed per mole biomass synthesized.

For respiring microorganisms, the redox enzyme E consumed during biomass synthesis can be replenished by coupling the reduction of coenzyme E^+ to the oxidation of electron donor D (that is reaction 39). The overall reaction in this case becomes

$$\frac{5z}{n} \sum_D \nu_D D + 5CH_pO_q + \nu_P^X ATP + NH_4^+ + (\nu_P^X + 2 - 5q)H_2O + (5z - 2\nu_P^X - 1)H^+$$
$$\rightarrow \frac{5z}{n} \sum_{D^+} \nu_{D^+} D^+ + C_5H_7O_2N + \nu_P^X ADP + \nu_P^X P_i \tag{55}$$

the sum of reactions 39 and 54. Here $\nu_P^X = \nu_P^S - (5z/n)\nu_P$ represents the net number of ATPs consumed per biomass synthesized. In theory, we can calculate the thermodynamic driving force f_X for biomass synthesis
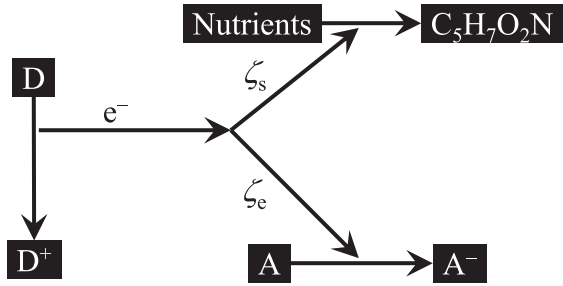
Fig. 3. Division of electrons between respiration and biomass synthesis. Electrons are released from the oxidation of electron donor D to its oxidized form D^+. A fraction ζ_e of electrons released is consumed by reducing the electron acceptor A to its reduced form A^-. The remainder $\zeta_s = 1 - \zeta_e$ is consumed by biomass synthesis, represented here as the conversion of nutrients to biomass, $C_5H_7O_2N$.

$$f_X = -\Delta G_X^R + \nu_P^X \cdot \Delta G_P \tag{56}$$

as the difference between the negative of the Gibbs free energy change ΔG_X^R of the oxidation of electron D (reaction 31) coupled to biomass synthesis half-reaction (reaction 53) and the energy available from hydrolyzing ATP ($\nu_P^X \cdot \Delta G_P$). For most microorganisms, however, appropriate values for ΔG_X^R and ν_P^X remain to be determined and the driving force for biomass synthesis cannot be evaluated to this level of precision.

Microbial Metabolism

A microbe's metabolism is the sum of its catabolism (that is respiration or fermentation) and its anabolism (that is biomass synthesis). Where the reduction degree of the carbon source is less than that of the biomass (that is $\gamma_C < \gamma_B$), catabolism and anabolism both consume electrons released from the oxidation of the electron donor (fig. 3) or fermented organic compound. The fractions of electrons consumed by catabolism and anabolism are denoted ζ_e and ζ_S, respectively (McCarty, 1971); the two fractions sum to one, that is $\zeta_e + \zeta_S = 1$.

The metabolism of a respiring microorganism has commonly been represented as the result of a single reaction, by adding the product of ζ_e and respiration (reaction 44) and the product of ζ_S and biomass synthesis (reaction 55) (for example, Stouthamer and Van Verseveld, 1985; VanBriesen and Rittmann, 2000, *et cetera*). Expressing microbial metabolism in this way suggests microbial catabolism and anabolism are fully coupled and, as a result, the reaction coefficients for substrate consumption and biomass synthesis remain invariant regardless of the changes in the environment. In fact, microbial catabolism and anabolism are parallel and independent metabolic pathways and microbial metabolism is just a convenient term to summarize the ensemble of biochemical reactions within a cell. A microorganism regulates catabolism and anabolism simultaneously to achieve its goal of survival and growth, but there is no evidence suggesting an enzymatic coupling between catabolism and anabolism or therefore a single reaction describing its metabolism.

The values of ζ_e and ζ_S reflect the partitioning of electrons between catabolism and anabolism. It is worth noting that ζ_e and ζ_S do not affect the thermodynamic driving forces f for respiration (eq 50), because respiration and biomass synthesis are parallel reactions in a microbe's metabolism. The thermodynamic driving force f is by definition the chemical energy available to drive respiration, or the electron transfer from the oxidation of electron donor D to the reduction of acceptor A. An electron

consumed in the half-reaction of biomass synthesis (reaction 53) does not participate in respiration and, therefore, does not affect the driving force for respiration.

<div align="center">THERMODYNAMIC POTENTIAL FACTOR</div>

The rates of microbial fermentation, respiration, and biomass synthesis vary directly with the thermodynamic potential factor, according to equation (17). For microbial fermentation and respiration, the factor can be written as

$$F_T = 1 - \exp\left(-\frac{\Delta G_A - \Delta G_C}{\chi RT}\right) \tag{57}$$

by substituting equation (30) and (50) into (18). Comparing this relation to the thermodynamic term commonly carried in rate laws for abiotic reactions (eq 2), we see an additional term of the energy conserved (ΔG_C), which takes into account that microbial metabolism conserves energy from the environments. In other words, the energy conserved ΔG_C is not available to drive the metabolism forward.

Average Stoichiometric Number

The average stoichiometric number χ can in principle be determined from the mechanism of the overall metabolic reaction as well as the Gibbs free energy change or driving force for each elementary step. This information, however, is unavailable for most metabolisms. Instead, we can assume that a single rate determining (or limiting) step controls progress of the overall reaction. At steady state, the thermodynamic drive for other steps is insignificant compared to that for the rate determining step. In other words, we can approximate the thermodynamic driving force f for the overall reaction as

$$f = \chi_{rd}f_{rd} + \sum_{i\neq rd,i=1}^{N-1} \chi_i f_i \approx \chi_{rd}f_{rd} \tag{58}$$

and the sum of the driving forces f_i for each step as

$$\sum_{i=1}^{N-1} f_i = f_{rd} + \sum_{i\neq rd,i=1}^{N-1} f_i \approx f_{rd} \tag{59}$$

where χ_{rd} is the number of times that the rate determining step takes place per turnover of the overall reaction and f_{rd} is the step's driving force. Substituting equations (58) and (59) into (16), we see that $\chi = \chi_{rd}$, that is, the average stoichiometric number χ is approximately χ_{rd}.

Since the number of times the rate determining step occurs in the overall reaction depends on how the reaction (for example, reactions 29 and 44) is written, so does the value of χ (Jin and Bethke, 2005). If the stoichiometric coefficients (those are ν_S, ν_P, ν_D, ν_A, *et cetera*) of reactions 29 and 44 were to double, the value of χ_{rd} would double as well, and so will the average stoichiometric number χ. For microbial catabolism, likely rate determining steps are ATP synthesis during fermentation, proton translocation, substrate activation (for example, benzoyl-CoA formation), electron transfer to extracellular electron acceptors, and so on.

Thermodynamic Control

The thermodynamic potential factor varies as a function of the thermodynamic driving force, as shown in figure 4. The driving force is large where the energy available to a microbe from its environment, ΔG_A, is much greater than that conserved by
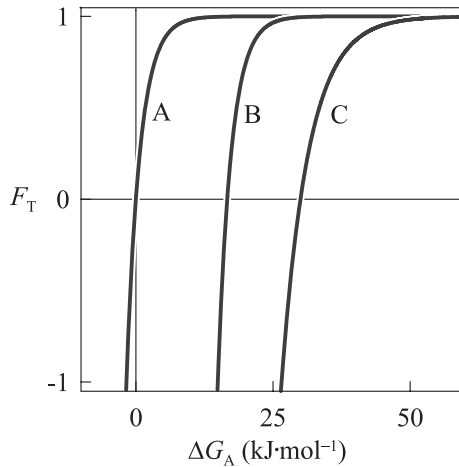
Fig. 4. Variation of the thermodynamic potential factor F_T with the energy available ΔG_A. Line A and B represent previous models (those are eq 62 and 64, respectively). Line C represents the model we present (eq 57), taking a value for the average stoichiometric number χ of 2 and that for energy conserved ΔG_C as 30 kJ $\cdot$ mol^{-1}. In evaluating equation (64), the phosphorylation potential ΔG_P is taken as 50 kJ $\cdot$ (mol ATP)$^{-1}$.

microbial metabolism, ΔG_C. Under such conditions, the value of F_T approaches unity and the metabolic rate r, the product of F_T and the forward reaction rate r_+, is about the same as the forward rate. Taking χ to be two, a driving force of 15 kJ $\cdot$ mol^{-1} translates to a value for F_T of about 0.95, for example, and the net reaction rate is therefore about 95 percent of the forward rate. Where the driving force is large, therefore, the thermodynamic potential factor plays only a small role in determining the metabolic rate and can commonly be neglected.

The thermodynamic potential factor, conversely, can exert a strong control on metabolic rate if the thermodynamic drive is small. Where the energy available (ΔG_A) falls close to that conserved by microbial metabolism (ΔG_C), the driving force approaches zero and so does the value of F_T. As a result, microbial metabolism proceeds at a net rate much smaller than the forward reaction rate. In natural environments, the driving force for biogeochemical processes such as sulfate reduction, methanogenesis, *et cetera*, can be quite small. The small drives result from the fact that such chemical reactions commonly liberate little free energy under standard conditions (that is $-\Delta G°$ is small, or even negative), and because the supply of substrates in natural environments can be severely depleted. For many biogeochemical processes, therefore, the thermodynamic potential factor has to be considered to predict their rates.

The value of F_T assumes a zero value where the driving force vanishes, as is the case where the energy available in the environment balances the energy conserved by the microbe. The cessation of microbial metabolism once the energy available falls to a finite value has been widely reported for experimental studies of the fermentation of ethanol, propionate, butyrate, and benzoate (Seitz and others, 1990; Wallrabenstein and Schink, 1994; Westermann, 1994; Wu and others, 1994; Hopkins and others, 1995; Warikoo and others, 1996; Scholten and Conrad, 2000; Jackson and McInerney, 2002; Jin, 2007) and for many types of microbial respiration, including nitrate reduction (Cord-Ruwisch and others, 1988; Seitz and others, 1990; Lu and others, 2001), arsenate reduction (Blum and others, 1998; Jin and Bethke, 2003), iron reduction (Liu and others, 2001; Roden and Urrutia, 2002; Dominik and Kaupenjohan, 2004; He and Sanford, 2004), sulfate reduction (Cord-Ruwisch and others, 1988; Seitz and others,

1990; Hoehler and others, 1998, 2001; Sonne-Hansen and others, 1999), methanogenesis (Jetten and others, 1990; Westermann, 1994; Yang and McCarty, 1998; Hoehler and others, 1998, 2001), and acetogenesis (Conrad and Wetter, 1990; Peters and others, 1998).

At the point at which the thermodynamic drive vanishes,

$$\Delta G_C = \Delta G_A \tag{60}$$

The amount of energy available in an experiment at the point at which metabolism ceases, then, is a measure of the amount of energy a microbe conserves. This observation provides a convenient approach for estimating energy conservation by fermenting and respiring microbes, even in cases where details of the metabolic pathway have yet to be resolved.

A driving force less than zero occurs where insufficient energy is available to support energy conservation. In this case, F_T becomes negative, suggesting that microbial metabolism proceeds in reverse. Microbial catabolism in the reverse direction would consume rather than conserve energy, wasting a cell's energy store. To avoid dissipation of its energy stores, a microorganism will normally regulate the activities of the enzymes in its respiratory chain in order to stop the metabolism. We can, therefore, represent the rate of microbial metabolism in a form

$$r = \begin{bmatrix} r_+\left[1 - \exp\left(-\dfrac{\Delta G_A - \Delta G_C}{\chi RT}\right)\right]; & \Delta G_A > \Delta G_C \\ 0; & \Delta G_A \le \Delta G_C \end{bmatrix} \tag{61}$$

that accounts for the possibility of a negative thermodynamic drive and the necessity of a microbe, under such conditions, preserving its energy stores.

Other Models

We can compare the thermodynamic potential factor proposed herein (eq 61) to previous attempts to honor thermodynamic consistency in the description of microbial metabolism (fig. 4). Hoh and Cord-Ruwisch (1996) proposed a thermodynamic term of the form

$$F' = 1 - \exp\left(-\frac{\Delta G_A}{RT}\right) \tag{62}$$

We see that this factor is a simplification of the thermodynamic potential factor for the case in which microbial metabolism is an elementary process (that is $\chi = 1$) that doesn't conserve energy ($\Delta G_C = 0$). A factor of this form suggests that microbial metabolism would proceed until the chemical reaction (for example, reactions 25 and 37) reaches a thermodynamic equilibrium, regardless whether or not the driving force is positive.

A second model (Kleerebezem and Stams, 2000) recognizes that a microorganism can utilize only part of the energy available to drive its metabolic reaction forward. These authors modified the model of Hoh and Cord-Ruwisch (1996) as

$$F' = 1 - \exp\left(-\frac{\Delta G_A - \Delta G_C}{RT}\right) \tag{63}$$

to account for the thermodynamic drive for the fermentation of butyrate to dihydrogen and acetate. This model represents a special case of equation (61) in which χ takes a value of 1.

A third model (Liu and others, 2001) is based on the concept of a biological energy quantum (Schink and Friedrich, 1994). According to this concept, ATP synthase produces one ATP for every three protons translocated inside the cell membrane. The minimum quantum of energy, therefore, is the energy required to translocate one proton out of cell membrane, about one third of energy required for ATP synthesis. In other words, for microbial metabolism to proceed forward, the energy available should be larger than one third of the energy required for ATP synthesis. Taking into account this minimum quantum energy, Liu and others (2001) proposed a factor of

$$F' = 1 - \exp\left(-\frac{\Delta G_A - \frac{1}{3}\Delta G_P}{RT}\right) \tag{64}$$

According to this model, microbial metabolism proceeds until the energy available decreases to one third of the phosphorylation potential. Comparing this relation to equation (61), we see that this model holds for the special case in which microbial metabolism is an elementary process (that is $\chi = 1$) that produces one-third of an ATP per turnover of the reaction.

Curtis (2003) modified the model of Hoh and Cord-Ruwisch (1996) so that

$$F' = \begin{cases} 1 - \exp\left(-\frac{\Delta G_A}{RT}\right), & \Delta G_A > \Delta G_{THR} \\ 0, & \Delta G_A \leq \Delta G_{THR} \end{cases} \tag{65}$$

In this way, they provided for the cessation of microbial metabolism where the energy available decreases below a threshold ΔG_{THR}. Comparing this model to equation (61), we see the threshold energy ΔG_{THR} is in fact the amount of energy ΔG_C conserved, which is more properly subtracted from the energy available ΔG_A to give the thermodynamic control.

RATE LAW

The rate r_F of microbial fermentation (reaction 25) is the number of moles of the substrate S degraded per unit volume, per unit time ($mol \cdot liter^{-1} \cdot sec^{-1}$, or $M \cdot sec^{-1}$)

$$r_F = -\frac{d[S]}{dt} = \frac{d[P^+]}{\nu_{P+}dt} = \frac{d[P^-]}{\nu_{P-}dt} \tag{66}$$

The rate r_R of microbial respiration (reaction 37) is the mole number of electrons transferred through the respiratory chain per unit volume, per unit time

$$r_R = -\frac{d[D]}{\nu_D dt} = -\frac{d[A]}{\nu_A dt} = \frac{d[D^+]}{\nu_{D+}dt} = \frac{d[A^-]}{\nu_{A-}dt} \tag{67}$$

And, the rate r_X of biomass synthesis (reaction 55) is the number of grams of biomass synthesized per unit volume, per unit time ($g \cdot liter^{-1} \cdot sec^{-1}$)

$$r_X = \frac{d[X]}{dt} \tag{68}$$

Here [X] is biomass concentration (g biomass $\cdot liter^{-1}$, or $g \cdot liter^{-1}$). A general kinetic theory for microbial metabolism requires laws be developed describing each of these rates.

Steady State

At steady state, the energy conserved (per unit volume per unit time) during catabolism equals that consumed by biomass synthesis and cell maintenance. For a respiring microorganism, the energy conservation rate r_R^E (kJ $\cdot$ liter^{-1} $\cdot$ sec^{-1}) is

$$r_R^E = \nu_P^{R'} \cdot \Delta G_P \cdot r_R / n \tag{69}$$

the product of the energy conserved per mole of electrons transferred and the rate of electron transfer. The rate r_X^E of energy consumption during biomass synthesis is

$$r_X^E = \nu_P^X \cdot \Delta G_P \cdot \frac{r_X}{w} \tag{70}$$

which is the product of energy consumed per gram of biomass synthesized and the rate of biomass synthesis. Here, w, the molecular weight for biomass. The rate r_M^E of energy consumption during cellular maintenance can be expressed

$$r_M^E = [X] \cdot r_M \cdot \Delta G_P \tag{71}$$

where r_M is the number of ATPs consumed in maintaining each gram of biomass per unit time. Cellular maintenance refers to a variety of biochemical processes that do not contribute to biomass synthesis and microbial growth, such as maintaining the proton motive force, transporting solutes across the cell membrane, the turnover of cellular macromolecules, cell motility, *et cetera* (Neidhardt and others, 1990). At steady state,

$$r_R^E = r_X^E + r_M^E \tag{72}$$

Substituting equations (69), (70), and (71), we see that

$$r_X = Y \cdot r_R - D \cdot [X] \tag{73}$$

where $Y = w \cdot \nu_P^{R'} / (\nu_P^X \cdot n)$ is the growth yield (g $\cdot$ mol^{-1}), the grams of biomass synthesized per mole of electrons transferred through the respiratory chain, and $D = w \cdot r_M / \nu_P^X$ has been termed the specific maintenance rate (sec^{-1}) (Marr and others, 1962; Pirt, 1965). Equation (73) was developed empirically by Herbert (1958) to account for the loss of biomass due to cellular maintenance. According to equation (73), although microbial catabolism and anabolism are not coupled by any enzymes, the balance between the energy production and consumption at steady state gives rise to a linear relationship between the rates of biomass synthesis and those of respiration (or fermentation). To predict the rate of microbial metabolism (catabolism and anabolism) at steady state, we need only predict the rate of microbial respiration (or fermentation) and then subtract the rate of biomass synthesis, calculated according to equation (73).

Once the rates of microbial respiration (or fermentation) and biomass synthesis are known, we can predict the rates at which the chemical species involved in microbial metabolism are consumed or produced. For a respiring microorganism utilizing a carbon source with reduction degrees less than that of biomass, both respiration and biomass synthesis consume the electron donor D (reactions 44 and 55). The rate of electron donor consumption can be calculated

$$\frac{d[D]}{dt} = -\nu_D \cdot r_R - \frac{5 \cdot z \cdot \nu_D}{n \cdot w} r_X \tag{74}$$

as the sum of the rates at which respiration and biomass synthesis consume the electron donor.

Forward Rate

From equation (61), we see that a thermodynamically consistent rate law for microbial fermentation and respiration is composed of two parts: the thermodynamic potential factor (eq 61) and the forward reaction rate r_+. To predict the metabolic rate, we need a description of the latter. Various empirical rate laws, such as the zero-order, first-order, logistic, logarithmic, Monod, and dual-Monod equation, have been proposed to describe respiration and fermentation (for example, Monod, 1949; Megee and others, 1972; Berner, 1980; Lee and others, 1984; Simkins and Alexander, 1984; Bae and Rittmann, 1996; *et cetera*).

We can generalize these simple laws as a multi-Monod equation (Jin and Bethke, 2005)

$$r_+ = k[\text{X}] \prod_{\text{C}} F_{\text{C}} \qquad (75)$$

where k is the rate constant (mol $\cdot$ (mg $\cdot$ sec)$^{-1}$) and F_{C} are kinetic factors

$$F_{\text{C}} = \frac{[\text{C}]}{[\text{C}] + K_{\text{C}}} \qquad (76)$$

that describe how the concentration [C] of the organic substrate controls the fermentation rate, or the concentration of electron donor or acceptor controls the respiration rate. Here, K_{C} is the half-saturation constant (mol $\cdot$ liter^{-1} or molal). These laws predict well the forward rates of fermentation and respiration when applied under appropriate geochemical conditions, such as large substrate concentrations, buffered pH, and no buildup of metabolic products.

By combining a simple law of such form with the thermodynamic potential factor, we can generalize the rate law to apply over a range of thermodynamic conditions. In other words, appending the thermodynamic factor produces a modified rate law that honors the requirements of thermodynamic consistency. A general rate law predicting the rate r_{F} of the microbial fermentation

$$r_{\text{F}} = \begin{cases} k[\text{X}] \prod_{\text{S}} \dfrac{[\text{S}]}{[\text{S}] + K_{\text{S}}} \left[1 - \exp\left(-\dfrac{f}{\chi RT} \right) \right]; & f > 0 \\ 0; & f \leq 0 \end{cases} \qquad (77)$$

can be written from equations (61), (75), and (76), where K_{S} is the half-saturation constant for the substrate and the driving force f is calculated according to equation (30). The respiration rate r_{R} can be predicted according to

$$r_{\text{R}} = \begin{cases} k[\text{X}] \dfrac{\Pi_{\text{D}}[\text{D}]}{\Pi_{\text{D}}[\text{D}] + K_{\text{D}}} \cdot \dfrac{\Pi_{\text{A}}[\text{A}]}{\Pi_{\text{A}}[\text{A}] + K_{\text{A}}} \left[1 - \exp\left(-\dfrac{f}{\chi RT} \right) \right]; & f > 0 \\ 0; & f \leq 0 \end{cases} \qquad (78)$$

where K_{D} and K_{A} are half-saturation constants for electron donor D and acceptor A, respectively, and the driving force is calculated according to equation (50).

Where the energy available is abundant and the thermodynamic drive is large, as we have noted, the value of F_{T} in the general rate law (eq 77 and eq 78) approaches unity and forward reaction overwhelms reverse; as such, the general rate law simplifies to the simple laws (eq 75). For example, laboratory studies, in order to promote microbial growth, are commonly conducted in the presence of a large thermodynamic driving force. This is also the case where microbial metabolism in the environments is

driven by abundant energy available, such as organic compounds exposed to atmospheric oxygen.

We have shown previously that the thermodynamic driving force can exert a significant control on the rate of microbial respiration (Jin and Bethke, 2003, 2005). In this section, we demonstrate how thermodynamics can control the progress of enzymatic reactions and the metabolism of fermentative micororganisms.

Enzymatic Reaction

Syntrophus gentianae can ferment benzoate syntrophically, relying on sulfate-reducing bacteria and methanogens to consume the dihydrogen it produces (Schink, 1992). During fermentation, benzoate-CoA ligase combines benzoate and coenzyme A (CoASH) to produce benzoyl-CoA

$$\text{Benzoate} + \text{CoASH} + \text{H}^+ \leftrightarrows \text{Benzoyl-CoA} + \text{H}_2\text{O} \qquad (79)$$

The Gibbs free energy change ΔG is

$$\Delta G = \Delta G^{o'} + RT \ln \frac{[\text{Benzoyl-CoA}]}{[\text{Benzoate}][\text{CoASH}]} \qquad (80)$$

Here the standard Gibbs free energy change at pH 7, $\Delta G^{o'}$, takes a value of 25.0 kJ · (mol benzoate)$^{-1}$ at 25°C (Thauer and others, 1977; Schöcke and Schink, 1999). Where concentrations of benzoate, benzoyl-CoA, and coenzyme A are in similar mM ranges, the value of ΔG remains positive and reaction 79 should proceed backwards, decomposing benzoyl-CoA to benzoate and coenzyme A.

Benzoate-CoA ligase catalyzes the forward reaction by coupling it to ATP hydrolysis to form AMP and PP$_i$ (reaction 21). Since one ATP is hydrolyzed per benzoyl-CoA produced, the overall reaction can be represented

$$\text{Benzoate} + \text{CoASH} + \text{ATP} \leftrightarrows \text{Benzoyl-CoA} + \text{AMP} + \text{PP}_i + \text{H}^+ \qquad (81)$$

as the sum of reactions 79 and 21. According to equation (24), the thermodynamic driving force for this reaction is

$$f = -\Delta G - \Delta G_M \qquad (82)$$

which is the negative Gibbs free energy change for reaction 81. Here, ΔG is the Gibbs free energy for reaction 79 (eq 80).

Schöcke and Schink (1999) studied the progress of benzoyl-CoA formation catalyzed by benzoate-CoA ligase purified from *S. gentianae* (fig. 5). In their experiments, a cell-free extract containing benzoate-CoA ligase was added to a pH-buffered solution containing 1 mM benzoate, 1 mM coenzyme A, and 1 mM ATP. With time, the concentrations of benzoate, coenzyme A, and ATP decreased, while benzoyl-CoA and AMP accumulated. After about 10 minutes, the concentrations of these species held constant, indicating the reaction had ceased. At this point, 0.65 mM of benzoate, 0.47 mM of coenzyme A, and 0.65 mM of ATP remained in the system.

To test whether the thermodynamic driving force (eq 82) controls the rate of benzoyl-CoA formation, we need to evaluate how the Gibbs free energy change ΔG of reaction 79 (eq 80) and the energy ΔG_M released by ATP hydrolysis (reaction 21) vary over the course of the experiment. As shown in figure 6, the value of ΔG, about 32.2 kJ · (mol benzoate)$^{-1}$ at the beginning of experiment, increases with time, approaching a constant value of 42.5 kJ · (mol benzoate)$^{-1}$. The increase is due to the rise in benzoyl-CoA concentration and declines in the concentrations of benzoate and
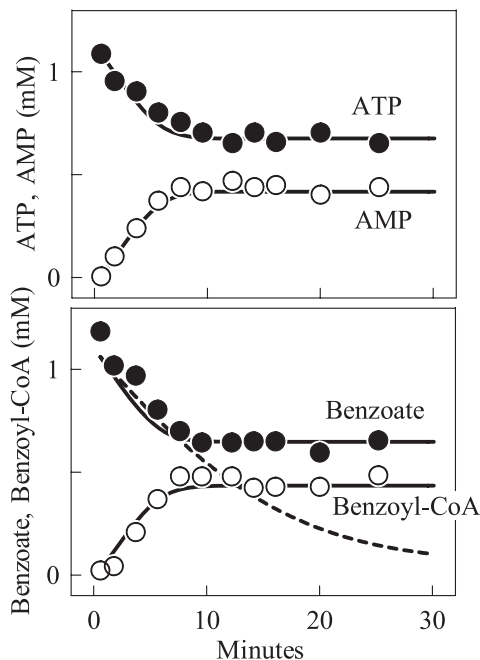
Fig. 5. Results of an experimental study of benzoate activation to benzyol-CoA by benzoate-CoA ligase extracted from *Syntrophus gentianae*, as reported by Schöcke and Schink (1999, their fig. 1). Lines are concentrations of benzoate, benzoyl-CoA, ATP, and AMP predicted by integrating the modified Michaelis-Menten equation (eq 85). The dashed line is the benzoate concentration predicted by the simple form of the Michaelis-Menten equation (eq 84), neglecting the thermodynamic control.

coenzyme A (eq 80). The value of ΔG_M, about $-63.8\,\text{kJ} \cdot (\text{mol ATP})^{-1}$ at the beginning of experiment, becomes less negative with time as ATP is consumed and the reaction products AMP and PP_i build up. After 10 minutes, the value of ΔG_M approaches $-42.5\,\text{kJ} \cdot (\text{mol ATP})^{-1}$.

The thermodynamic driving force *f*, the difference between $-\Delta G$ and ΔG_M (eq 82), is about $31.6\,\text{kJ} \cdot (\text{mol benzoate})^{-1}$ at the beginning of the experiment. The value decreases as ΔG and ΔG_M increase, reaching zero when the Gibbs free energy change ΔG_M of ATP hydrolysis balances that of benzoyl-CoA formation (ΔG). At this point, the rate of benzoyl-CoA formation decreases to zero and reaction 81 ceases. The reaction stops not because of the lack of benzoate, CoASH, or ATP, all of which remain in the experiment at significant concentration, but due to the strong thermodynamic control on the reaction's progress.

To catalyze reaction 81, benzoate-CoA ligase first combines with ATP and then benzoate to form a benzoate-ATP-enzyme complex. ATP is then hydrolyzed and pyrophophate is released. Coenzyme A then combines with the benzoate-AMP-enzyme complex to produce benzoyl-CoA. After benzoyl-CoA is released from the enzyme complex, AMP is also released, returning the enzyme to its native state (Vessey and Kelley, 2001). Such a linear sequential reaction mechanism indicates that the rate determining step occurs once per benzoyl-CoA produced. The average stoichiometric number χ for reaction 81 thus takes a value of 1.

We can quantify the thermodynamic control on the reaction rate using the thermodynamic potential factor F_T

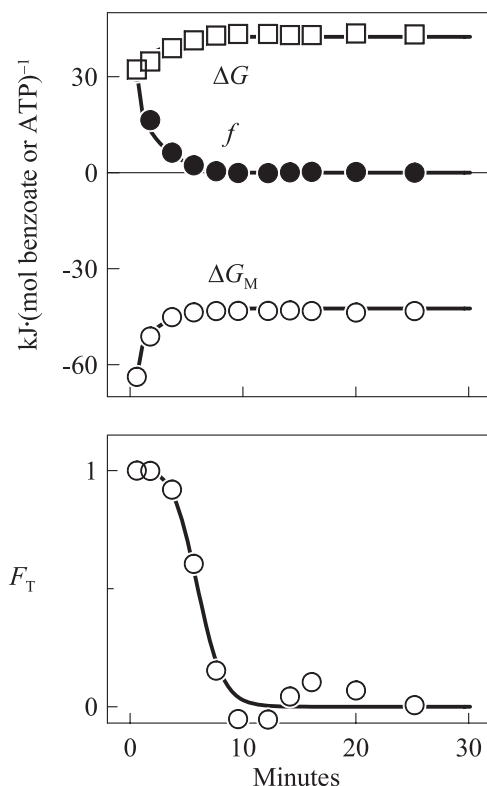$$F_T = 1 - \exp\left(\frac{\Delta G + \Delta G_M}{RT}\right) \tag{83}$$

Fig. 6. Variation with time of the Gibbs free energy change (ΔG), the free energy released from ATP hydrolysis to AMP and PP_i (ΔG_M), thermodynamic driving force f, and the thermodynamic potential factor F_T in the experiment study of benzoyl-CoA formation (Schöcke and Schink, 1999). Lines are values of ΔG_M, ΔG, f, and F_T calculated using equation (22), (80), (82), and (57), respectively. Data points are the results calculated directly from the reported experiment data.

by substituting equation (82) into (18). As shown in figure 6, the value of F_T, about unity at the beginning of the experiment, decreases with time due to the decreases in the driving force f. When the driving force disappears after about 10 minutes, the value of F_T decreases to zero and, according to the thermodynamically consistent rate law (eq 17), so does the predicted reaction rate .

   As discussed above, we can account for the thermodynamic control by appending the thermodynamic potential factor F_T (eq 57) to the rate law describing the forward progress of reaction 81. In enzyme kinetics, the forward rate is often described by the Michaelis-Menten equation (Plowman, 1972). For reaction 81, which consumes multiple substrates, we can describe the forward rate as the product of the Michaelis-Menten equations (Schöcke and Schink, 1999) written for each substrate individually,

$$r = r_{max} \frac{[\text{Benzoate}]}{[\text{Benzoate}] + K_{Ben}} \frac{[\text{CoASH}]}{[\text{CoASH}] + K_{CoA}} \frac{[\text{ATP}]}{[\text{ATP}] + K_{ATP}} \tag{84}$$

where r_{max} is the maximum rate for a given amount of benzoyl-CoA ligase (mol · liter^{-1} · sec^{-1}), and K_{Ben}, K_{CoA}, and K_{ATP} are the Michaelis-Menten constants for benzoate, CoASH, and ATP, respectively. The thermodynamically consistent form of this equation is
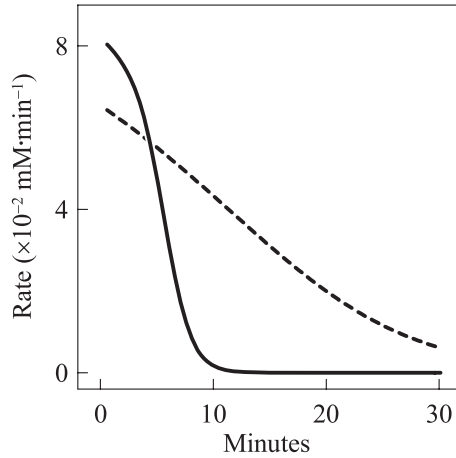
Fig. 7. Variation of reaction rate with time predicted for the experimental study of benzoyl-CoA formation (Schöcke and Schink, 1999). Solid line represents reaction rate calculated using the modified Michaelis-Menten equation (eq 85); the dashed line show the rate calculated according to the Michaelis-Menten equation (eq 84).

$$r = r_{max} \frac{[\text{Benzoate}]}{[\text{Benzoate}] + K_{Ben}} \frac{[\text{CoASH}]}{[\text{CoASH}] + K_{CoA}} \frac{[\text{ATP}]}{[\text{ATP}] + K_{ATP}} F_T \qquad (85)$$

where F_T is given by equation (83).

We can predict how the concentrations of chemical species vary with the experiment progress by integrating equation (85) numerically. In evaluating the rate law, we take a value of 2.5×10^{-6} mol $\cdot$ (liter $\cdot$ sec)$^{-1}$ for r_{max}. The values for K_{Ben}, K_{CoA}, and K_{ATP}, as determined by Schöcke and Schink (1999), are 0.026 mM, 0.8 mM, and 0.05 mM, respectively. We assume the enzyme activity, and also the value of r_{max}, remains constant throughout the experiment since the experiment duration of 25 minutes is relatively short and the experimental conditions unchanged. As shown in figure 5, the modeling results fit the observations well. The concentrations of benzoate and ATP decrease for about 10 minutes, then remain constant. Figure 7 shows how the rate calculated by equation (85) varies over the course of the experiment. The rate is at its maximum at the beginning of the experiment and decreases to zero after 10 minutes.

If we neglect the thermodynamic control and use the multiple Michaelis-Menten equation (eq 84) to predict the variations with time in the concentrations of chemical species, the results differ significantly from the laboratory observations as shown in figure 5. To best fit the experiment observations, we take a value of 2.0×10^{-6} mol $\cdot$ liter^{-1} $\cdot$ sec^{-1} for r_{max}. According to equation (84), the concentrations of benzoate, CoASH, and ATP decrease with time. Instead of approaching constant values after 10 minutes, however, the concentrations continue to decrease toward zero. Figure 7 shows how the rate calculated in this way varies with time. According to equation (84), the rate decreases with time, but remains non-zero over the entire experimental interval.

The differences between the predictions by the Michaelis-Menten equation (eq 84) and those by its revised form (eq 85) arise from the thermodynamic control on the rate of benzoyl-CoA formation. According to equation (84), the rate depends only on the concentrations of benzoate, coenzyme A, and ATP. Thus the calculated rate remains positive as long as the concentrations remain above zero, regardless whether the thermodynamic drive is positive or not. The thermodynamically consistent rate

equation (eq 85) takes into account how the thermodynamic driving force controls the rate. The rate vanishes when the driving force decreases to zero, even though significant amounts of substrates remain in the system to be catalyzed.

Crotonate Fermentation

The fermentation of crotonate ($CH_3CHCHCOO^-$) by *Syntrophus buswellii* provides a second example of how thermodynamic drive can affect the rate of microbial metabolism. *S. buswellii* can dismutate crotonate to acetate (CH_3COO^-) and butyrate according to

$$\text{Crotonate} + H_2O \rightleftarrows \text{Acetate} + \frac{1}{2}\text{Butyrate} + \frac{1}{2}H^+ \tag{86}$$
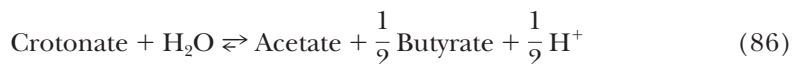
(Auburger and Winter, 1996). The Gibbs free energy change ΔG is

$$\Delta G = \Delta G^\circ + RT\ln\frac{[\text{Acetate}][\text{Butyrate}]^{1/2}[H^+]^{1/2}}{[\text{Crotonate}]} \tag{87}$$

The value of ΔG° at 25°C, calculated from the formation energies (Thauer and others, 1977), is -102.2 kJ · (mol crotonate)$^{-1}$.

On the basis of the mass balance between crotonate consumption and acetate and butyrate production observed in experiment studies (Wallrabenstein and Schink, 1994), we see that *S. buswellii* utilizes butyrate as a carbon source. The half-cell reaction for biomass synthesis (reaction 53) can be written

$$CH_3CH_2CH_2COO^- + \frac{4}{5}NH_4^+ \rightarrow \frac{4}{5}C_5H_7O_2N + \frac{2}{5}H_2O + \frac{19}{5}H^+ + 4e^- \tag{88}$$
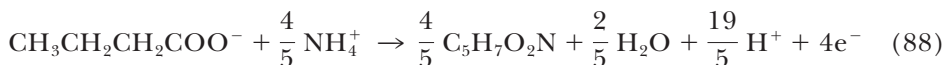
The reduction degree of butyrate is 5, higher than the value of 4 for biomass. As a result, biomass synthesis requires electron acceptors to proceed forward. The identity of the electron acceptor is unknown, but from the amount of biomass synthesized we can estimate the stoichiometric relationship between the crotonate fermented and butyrate produced. Auburger and Winter (1995) measured that about 9.5 g of biomass is produced per mol of crotonate fermented. In other words, about 0.08 mol of biomass ($C_5H_7O_2N$, M.W. 113.1) is synthesized per mol crotonate fermented, consuming 0.1 mol butyrate. For each mole of crotonate fermented, then, the mole numbers of acetate and butyrate produced are 1 and 0.4 (that is $0.5 - 0.1$), respectively.

Wallrabenstein and Schink (1994) studied crotonate fermentation by this strain at 28°C. Their culture was inoculated into batch reactors containing the growth medium with an initial concentration of 10 mM crotonate. With time, as shown in figure 8, the concentrations of biomass, acetate, and butyrate increase, while crotonate decreases. After about 16 days, crotonate concentration has fallen to a constant value of 0.5 mM, indicating fermentation has ceased.

Due to large crotonate concentration and low concentrations of acetate and butyrate at the beginning of the experiment, the energy initially available ($-\Delta G$, as given by eq 87) is considerable, about 70.0 kJ · (mol crotonate)$^{-1}$. As shown in figure 9, the energy decreases with time to 50.0 kJ · (mol crotonate)$^{-1}$ over about 16 days and remains constant thereafter.

On the basis of the observed growth yield, Auburger and Winter (1996) estimated that 0.9 ATPs are synthesized for each crotonate fermented. Assuming that the phosphorylation potential ΔG_P is 50 kJ · (mol ATP)$^{-1}$ and taking the number of ATPs synthesized per crotonate fermented as 1, the value of the energy conserved (ΔG_C) by *S. buswellii* is about 50.0 kJ · (mol crotonate)$^{-1}$. Assuming that the mechanism for crotonate fermentation does not vary over the course of the experiment, the value of
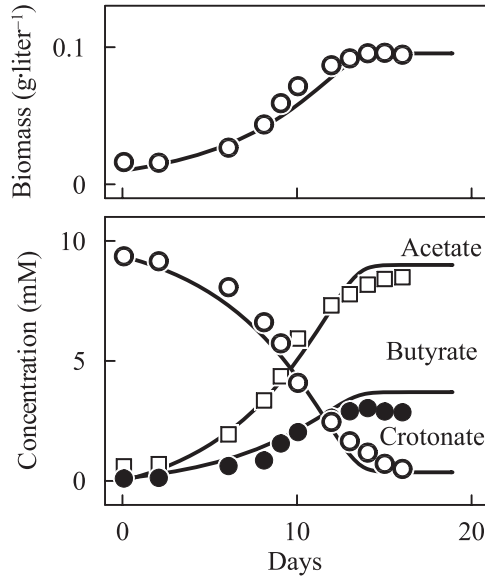
Fig. 8. Results of an experimental study of crotonate fermentation by *Syntrophus buswellii*, as reported by Wallrabenstein and Schink (1994, their figure 1). Solid lines are concentrations of biomass, crotonate, butyrate, and acetate predicted by integrating equations (73) and (91). Note that butyrate produced per crotonate fermented is only 0.34, less than 0.5 as shown in reaction 86, due to the compound's consumption as a carbon source for biomass synthesis.

ΔG_C can be taken to be constant. As shown in figure 9, the thermodynamic driving force f, the difference between $-\Delta G$ and ΔG_C, decreases from an initial value of about 20.0 kJ · (mol crotonate)$^{-1}$, reflecting the decrease in the energy available. After about 16 days, the energy available balances that conserved and the driving force falls to zero. Based upon the relatively small values of the thermodynamic driving force f for microbial fermentation, we see that the available energy controls significantly the rates of crotonate fermentation by *S. buswellii*.

To evaluate the thermodynamic control on the fermentation by *S. buswellii*, we need to estimate the value for the average stoichiometric number χ. During crotonate fermentation, *S. buswellii* activates crotonate to crotonyl-CoA, a step catalyzed by either crotonate:CoA ligase or CoA transferase (Auburger and Winter, 1996). Assuming crotonate activation is the rate determining step, the value of χ for the reaction written to yield a single crotonate is 1, since this step occurs once per crotonate fermented (reaction 86). The thermodynamic potential factor takes the form

$$F_T = 1 - \exp\left(\frac{\Delta G + \Delta G_C}{RT}\right) \tag{89}$$

As shown in figure 9, the value of F_T varies with the thermodynamic drive, starting near unity but, after about 16 days, falling sharply to about zero. The thermodynamic factor, then, becomes a significant factor controlling the rate of crotonate fermentation.

We can describe the forward fermentation rate using the Monod equation

$$r_+ = k[X]\frac{[\text{Crotonate}]}{[\text{Crotonate}] + K_S} \tag{90}$$
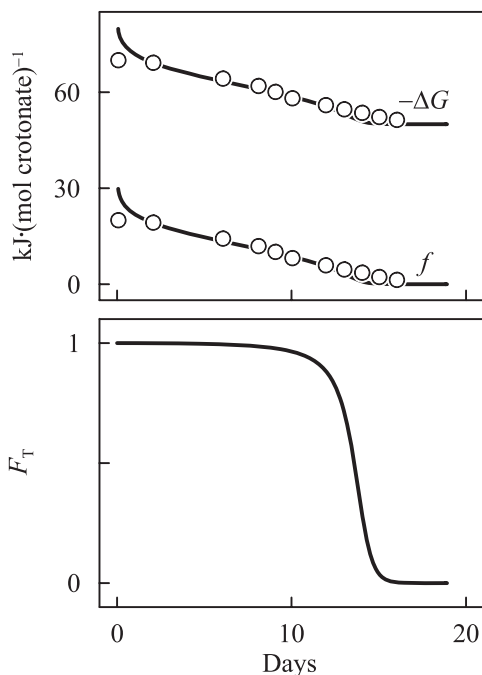
Adding the thermodynamic potential factor F_T,

Fig. 9. Variation with time of the energy available $(-\Delta G)$, thermodynamic driving force *f*, and thermodynamic potential factor F_T in the experiment study of crotonate fermentation (Wallrabenstein and Schink, 1994). Values of $-\Delta G$, *f*, and F_T are calculated using equations (87), (30), and (89), respectively. Data points are values calculated directly from the reported experimental data.

$$r = k[\text{X}] \frac{[\text{Crotoate}]}{[\text{Crotonate}] + K_S}\left[1 - \exp\left(\frac{\Delta G + \Delta G_C}{RT}\right)\right] \qquad (91)$$

gives a thermodynamically consistent form of the Monod equation. We model the experiment progress by integrating equations (73) and (91) numerically, taking into account the mole ratio of 0.4 for butyrate produced to crotonate fermented. In evaluating these equations, we take best-fit values of $20.0 \text{ mmol} \cdot \text{g}^{-1} \cdot \text{day}^{-1}$ for rate constant *k*, 0.5 mM for half-saturation constant K_S, $9.5 \text{ g} \cdot (\text{mol crotonate})^{-1}$ for growth yield *Y*, and zero for specific maintenance rate *D*. As shown in figure 8, the modified Monod equation (eq 91) predicts well the trend in crotonate concentration with time.

Figure 10 shows the rate of crotonate fermentation, as calculated using the modified Monod equation (eq 91). The rate predicted depends on the concentrations of biomass and crotonate, and the thermodynamic potential factor F_T. Crotonate concentration and F_T decrease with time, lowering the rate. The increasing biomass concentration, conversely, raises the rate. The net rate first increases with time due to the increasing biomass concentration, then, after about 12 days, starts to decrease in response to the thermodynamic control. The rate approaches zero after 16 days, along with the driving force and F_T.

If we were to neglect the thermodynamic control and use the simple Monod equation (eq 90) to model the experiment, the results predicted would deviate significantly from those observed (fig. 10). In this case, crotonate will continue to be metabolized until its concentration falls to zero. The Monod equation accounts only for the effects of the concentrations of crotonate and biomass and, as a result,
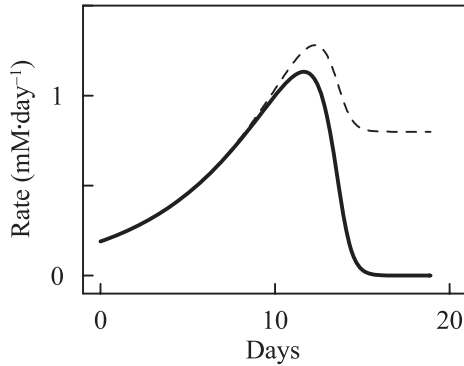
Fig. 10. Variation in fermentation rate with time as calculated for the experimental study of crotonate fermentation (Wallrabenstein and Schink, 1994). Solid line represents the reaction rate predicted using the modified Monod equation (eq 91); the dashed line represents the predictions by the Monod equation (eq 90), neglecting the thermodynamic control.

invariably predicts a positive rate even when the energy available is insufficient to drive the fermentation forward.
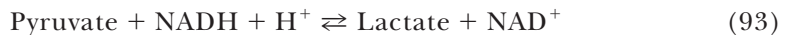
Glucose Fermentation

Microorganisms degrade natural organic matter in natural environments by hydrolyzing polymers such as polysaccharide to monomers like sugar molecules. The monomers are then fermented to dihydrogen, short-chain fatty acids, and so on. As a third example of the importance of thermodynamics in controlling metabolic rates, we consider how the thermodynamic drive controls the rate of glucose ($C_6H_{12}O_6$) fermentation. This example differs from the previous example in that the drive for the fermentation is considerably larger for glucose than crotonate.

Lactic acid bacteria can oxidize one glucose molecule to two pyruvate molecules through the glycolysis pathway, reducing two molecules of NAD^+ to NADH and synthesizing two ATPs

$$\text{Glucose} + 2NAD^+ \rightleftarrows 2 \text{ Pyruvate} + 2 \text{ NADH} + 4 \text{ H}^+ \tag{92}$$
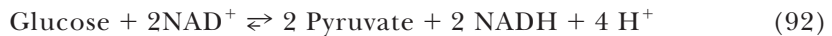
The NADH can be re-oxidized to NAD^+ by reducing pyruvate to lactate ($CH_3CHOHCOO^-$)

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \rightleftarrows \text{Lactate} + \text{NAD}^+ \tag{93}$$

The overall reaction for fermentation of glucose to lactate is

$$\text{Glucose} \rightleftarrows 2 \text{ Lactate} + 2 \text{ H}^+ \tag{94}$$

and the Gibbs free energy change is

$$\Delta G = \Delta G^\circ + RT \ln \frac{[\text{Lactate}]^2[\text{H}^+]^2}{[\text{Glucose}]} \tag{95}$$

The value of ΔG° at 25°C is -118.40 kJ $\cdot$ (mol glucose)$^{-1}$ (Thauer and others, 1977). Figure 11 shows how the energy available ($-\Delta G$) varies with glucose concentration, assuming in a fermentative environment pH of 3 and lactate concentration of 500 μM. Because $-\Delta G^\circ$ is large, the value of $-\Delta G$ is invariably positive (that is >150 kJ $\cdot$ (mol glucose)$^{-1}$) even at glucose concentrations as small as 1 μM. At glucose concentrations greater than 1 mM, the energy available is greater than 170 kJ $\cdot$ (mol glucose)$^{-1}$.
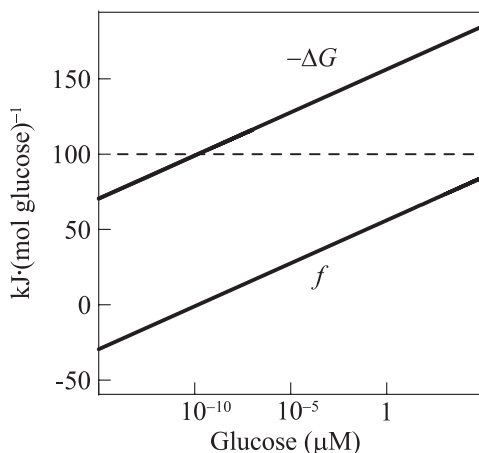
Fig. 11. Effect of glucose concentration on the energy available ($-\Delta G$, calculated according to eq 95) and driving force (f, by eq 30) for the example of glucose fermentation to lactate considered in the text. In evaluating equation (95), we assume a temperature at 25°C, pH of 3, and a lactate concentration of 500 μM. Dashed line represents the amount of energy conserved (that is 100 kJ · (mol glucose)$^{-1}$).

The amount ΔG_C of energy conserved can be determined from the number of ATPs produced during glycolysis (Stryer, 1988). Since two ATPs are synthesized per glucose fermented, the energy conserved $\Delta G_C = 2 \times \Delta G_P = 100$ kJ · (mol glucose)$^{-1}$, assuming a phosphorylation potential ΔG_P of 50 kJ · (mol ATP)$^{-1}$. Figure 11 shows how the thermodynamic driving force f, the difference between the energy available and the energy conserved, varies with glucose concentration. Since so much energy is available, the thermodynamic drive remains positive even at glucose concentrations as small as 10^{-5} μM. At glucose concentrations over 1 μM, the value of f increases to more than 50 kJ · (mol glucose)$^{-1}$.

We can assume that ATP synthesis is the rate determining step. During glycolysis, ATP synthesis step, either the formation of phosphoglycerate or that of pyruvate, occurs twice per glucose fermented. The average stoichiometric number χ therefore for the reaction written to produce one glucose takes a value of 2. The thermodynamic potential factor takes the form

$$F_T = 1 - \exp\left(\frac{\Delta G + \Delta G_C}{2RT}\right) \tag{96}$$

or, substituting equation (95),

$$F_T = 1 - \frac{[\text{Lactate}][\text{H}^+]}{[\text{Glucose}]^{1/2}} \exp\left(\frac{\Delta G^{\circ} + \Delta G_C}{2RT}\right) \tag{97}$$

in terms of glucose and lactate concentrations. Figure 12 shows how the value of F_T varies with glucose concentration under the conditions assumed above. Where glucose concentration is greater than 10^{-7} μM, the value of F_T remains close to unity. The value falls significantly below unity only for glucose concentrations below 10^{-7} μM. In other words, the thermodynamic control on glucose fermentation is significant only where glucose concentration is extremely small.

Since abundant energy is available to drive glucose fermentation, we can neglect the thermodynamic control under most circumstances and use the Monod equation
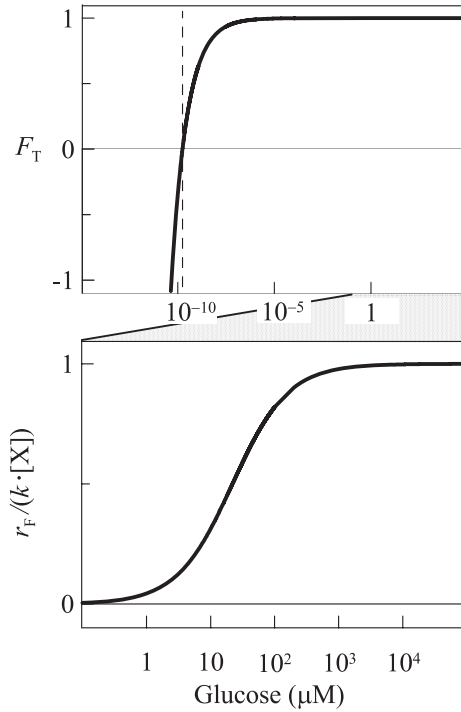
Fig. 12. Variation with glucose concentration of thermodynamic potential factor F_T (eq 96) and the relative metabolic rate $r_F/(k \cdot [X])$, the ratio of the fermentation rate r_F to maximum rate $(k \cdot [X])$, predicted by the Monod equation (eq 98) for the example of microbial metabolism fermenting glucose considered in text. K_S is taken to be 22 μM. Dashed line shows the glucose concentration where the value of the thermodynamic potential factor F_T crosses zero.

$$r_F = k[X] \frac{[\text{Glucose}]}{[\text{Glucose}] + K_S} \tag{98}$$

to describe reaction rate. Figure 12 shows how the relative rate $r_F/(k \cdot [X])$, the ratio of the fermentation rate r_F to maximum rate $(k \cdot [X])$, predicted by equation (98) varies with glucose concentration. In evaluating the rate law, we take K_S as 22 μM, as determined previously in laboratory experiments for *Escherichia coli* (Monod, 1949). F_T is near unity unless glucose concentration is extremely small, less than 10^{-7} μM. The fermentation rate, in contrast, varies strongly with glucose concentration at much higher glucose concentrations. The relative rate is less than one for glucose concentrations below about 1 mM, approaching zero at concentrations less than 1 μM.

The rates of biomass synthesis at steady state of the metabolism can be calculated by substituting equation (98) into (73),

$$r_X = k \cdot Y \cdot [X] \frac{[\text{Glucose}]}{[\text{Glucose}] + K_S} - D \cdot [X] \tag{99}$$

If we were to neglect the effect of cell maintenance on microbial growth, this equation would simplify to the original equation Monod proposed for the growth of fermenting microorganisms (Monod, 1949)

$$r_{\mathrm{X}} = r_{\mathrm{max}} \frac{[\mathrm{Glucose}]}{[\mathrm{Glucose}] + K_{\mathrm{S}}} \qquad (100)$$

We see the maximum rate r_{max} is the product of rate constant k for fermentation, biomass yield Y, and biomass concentration $[X]$. Microorganisms can also ferment glucose to acetate, formate, ethanol, *et cetera*. In each case, the energy available is large relative to the energy conserved, giving a large thermodynamic driving force. As a practical matter, then, the requirement of thermodynamic consistency need not be addressed in predicting the rate of glucose fermentation.

<div align="center">DISCUSSION</div>

In this paper we have cast study of the kinetics of microbial metabolism firmly within the framework of chemical thermodynamics, revealing a natural consistency not apparent in previous approaches to the problem. We have generalized our previous work on microbial respiration and extended it to describe processes of fermentation and enzyme catalysis, in each case developing a kinetic description wholly consistent with the principles of thermodynamics. We have shown how the requirement of thermodynamic consistency affects the form of kinetic rate laws governing microbial metabolism, and delineated the conditions under which honoring this requirement is important.

Rigorous analysis of the problem leads to a thermodynamic potential factor F_{T} (eq 57) that describes the thermodynamic control on the rate of microbial metabolism. The factor accounts for the availability of energy in the cell's environment, relative to that conserved as ATP and proton motive force. Whereas a rate law of traditional form is valid in the presence of abundant energy, appending F_{T} to the equation casts the law in a form applicable over a range of energetic conditions.

Geochemical environments differ broadly in their ability to supply chemical energy to microorganisms. In cases where the energy available far exceeds the energy conserved, the thermodynamic driving force is large and the thermodynamic potential factor approaches unity. In such cases, the thermodynamic control on microbial metabolism can be safely neglected, and the rate laws in common use work well. To figure the rate of a fermentative microorganism growing on glucose, for example, the thermodynamic potential factor can be neglected because the energy available is invariably large. Laboratory experiments, as a second example, are characteristically designed to supply abundant energy, in order to facilitate growth.

Many, perhaps most geochemical environments, in contrast, are less hospitable to microbes due to both the quality and quantity of substrates available to microorganisms. The quality of substrates may limit the energy available where the negative of the standard Gibbs free energy change of the reaction by which they are consumed is small. Microbial sulfate reduction and methanogenesis, for example, exploit chemical reactions that are commonly close to equilibrium and hence supply little energy to microorganisms. In many natural environments, furthermore, substrates may be present in only small quantities. The interiors of microbial mats and deep reaches of stratified water columns may be so depleted in electron acceptors or organic matter that little chemical energy can be derived there. In addition, the buildup of metabolic products in semi-closed environments can limit the amount of energy available. For example, many bacteria can oxidize short-chain fatty acids (propionate, butyrate, *et cetera*) and alcohol (ethanol, *et cetera*) by transferring electrons to hydrogen ions, reducing them to dihydrogen. Where dihydrogen accumulates in the environment, the energy available decreases, limiting the progress of microbial metabolism. In these cases, the thermodynamic potential factor assumes values considerably less than one, many times approaching zero or even becoming negative; the corresponding meta-

bolic rates, if the metabolism proceeds at all, may be quite small. Rate laws of traditional form, in contrast, invariably predict a positive rate, even where thermodynamics would drive metabolism backwards. It is of critical importance when considering microbes in natural environments, therefore, to appreciate that both kinetic and thermodynamic factors can exert significant controls on a microbe's metabolic rate.

APPENDIX

The relationship in the ratio of forward to reverse reaction rates between an overall reaction (reaction 3) and its elementary steps (reaction 4) (eq 12) can be derived by considering the forward and reverse passage of an individual molecule taking part in the overall chemical reaction (reaction 3). The probability of a molecule Sp_1 reacting to completion to form Sp_N over an interval of time Δt, denoted $P(Sp_1 \rightarrow Sp_N)$, is the product of the probabilities that each elementary step proceeds forward

$$P(Sp_1 \rightarrow Sp_N) = P(Sp_1 \rightarrow Sp_2) \cdot P(Sp_2 \rightarrow Sp_3) \cdot \quad \cdots \quad \cdot P(Sp_{N-1} \rightarrow Sp_N) \tag{A.1}$$

over this interval. Here, $P(Sp_i \rightarrow Sp_{i+1})$ represents the probability that an intermediate molecule Sp_i reacts over Δt to form Sp_{i+1}. Similarly, the probability that a molecule Sp_N reacts backward over Δt to form Sp_1

$$P(Sp_N \rightarrow Sp_1) = P(Sp_N \rightarrow Sp_{N-1}) \cdot P(Sp_{N-1} \rightarrow Sp_{N-2}) \cdot \quad \cdots \quad \cdot P(Sp_2 \rightarrow Sp_1) \tag{A.2}$$

is given as the product of the probabilities over this interval of a molecule following the reverse of each individual step.

Since r is the net rate of the overall reaction, the concentration of Sp_1 at time $t+\Delta t$ is $[Sp_1]^t - r \cdot \Delta t$, where $[Sp_1]^t$ is the concentration of Sp_1 at time t. For very small Δt, the number of Sp_1 per a unit volume over this interval is $[Sp_1]^t - r \cdot \Delta t/2$. During time interval Δt, if only one molecule of Sp_1 in a unit volume of fluid reacts to form one Sp_N, the probability of a particular molecule in the volume reacting to Sp_N is the inverse of the total number of Sp_1 in the volume, that is, $1/([Sp_1]^t - r \cdot \Delta t/2)$. Forward reaction occurs $r_+ \cdot \Delta t$ times over Δt, so $P(Sp_1 \rightarrow Sp_N)$ is given

$$P(Sp_1 \rightarrow Sp_N) = \frac{r_+ \cdot \Delta t}{[Sp_1]^t - r \cdot \Delta t/2} \tag{A.3}$$

Similarly, the probability that a given molecule Sp_N reacts to Sp_1 over Δt is

$$P(Sp_N \rightarrow Sp_1) = \frac{r_- \cdot \Delta t}{[Sp_N]^t + r \cdot \Delta t/2} \tag{A.4}$$

Here, $[Sp_N]^t$ is the concentration of Sp_N at time t. Combining equations (A.3) and (A.4), the ratio of $P(Sp_1 \rightarrow Sp_N)$ to $P(Sp_N \rightarrow Sp_1)$ is

$$\frac{P(Sp_1 \rightarrow Sp_N)}{P(Sp_N \rightarrow Sp_1)} = \frac{[Sp_N]^t + r \cdot \Delta t/2}{[Sp_1]^t - r \cdot \Delta t/2} \cdot \frac{r_+}{r_-} \tag{A.5}$$

For elementary step 1 ($Sp_1 \rightleftarrows Sp_2$), the probability that a given molecule Sp_1 reacts to Sp_2 over Δt, $P(Sp_1 \rightarrow Sp_2)$, can be estimated as we did for the overall forward reaction (eq A.3). If the step occurs χ_1 times per reaction turnover,

$$P(Sp_1 \rightarrow Sp_2) = \frac{\chi_1 \cdot r_{1+} \cdot \Delta t}{[Sp_1]^t - \chi_1 \cdot r_1 \cdot \Delta t/2} \tag{A.6}$$

where r_{1+} and r_1 are forward and net rates of step 1, respectively, and χ_1 is the step's stoichiometric number.

At steady state, the net rate r of overall reaction is the product of the step's net rate and stoichiometric number, that is $r = \chi_1 \cdot r_1$. Substituting gives

$$P(\text{Sp}_1 \rightarrow \text{Sp}_2) = \frac{\chi_1 \cdot r_{1+} \cdot \Delta t}{[\text{Sp}_1]^t - r \cdot \Delta t/2} \tag{A.7}$$

For the step $N-1$ ($\text{Sp}_{N-1} \rightleftarrows \text{Sp}_N$), similarly, the relation

$$P(\text{Sp}_N \rightarrow \text{Sp}_{N-1}) = \frac{\chi_{N-1} \cdot r_{(N-1)-} \cdot \Delta t}{[\text{Sp}_N]^t + r \cdot \Delta t/2} \tag{A.8}$$

gives the probability that a given molecule of Sp_N reacts over Δt to form Sp_{N-1}.

For an intermediate step i, since the intermediate's concentration $[\text{Sp}_i]$ remains constant at steady state, forward reaction occurs $\chi_i \cdot r_{i+} \cdot \Delta t$ times over Δt. The probability of an individual molecule participating in the forward reaction is

$$P(\text{Sp}_i \rightarrow \text{Sp}_{i+1}) = \frac{\chi_i \cdot r_{i+} \cdot \Delta t}{[\text{Sp}_i]} \tag{A.9}$$

For step $i-1$,

$$P(\text{Sp}_i \rightarrow \text{Sp}_{i-1}) = \frac{\chi_{i-1} \cdot r_{(i-1)-} \cdot \Delta t}{[\text{Sp}_i]} \tag{A.10}$$

is the probability of a given molecule Sp_i participating in the reverse reaction.

Substituting equations (A.7) and (A.9) into (A.1) gives the probability of a given molecule Sp_1 reacting to Sp_N

$$P(\text{Sp}_1 \rightarrow \text{Sp}_N) = \frac{\chi_1 \cdot r_{1+} \cdot \Delta t}{[\text{Sp}_1]^t - r \cdot \Delta t/2} \frac{\chi_2 \cdot r_{2+} \cdot \Delta t}{[\text{Sp}_2]} \cdots \frac{\chi_i \cdot r_{i+} \cdot \Delta t}{[\text{Sp}_i]} \cdots \frac{\chi_{N-1} \cdot r_{(N-1)+} \cdot \Delta t}{[\text{Sp}_{N-1}]}$$

$$= \frac{\chi_1 \cdot r_{1+} \cdot \Delta t}{[\text{Sp}_1]^t - r \cdot \Delta t/2} \prod_{i=2}^{N-1} \frac{\chi_i \cdot r_{i+} \cdot \Delta t}{[\text{Sp}_i]} \tag{A.11}$$

in terms of the forward reaction rates at each step. Substituting equations (A.8) and (A.10) into (A.2), gives the probability for the reverse reaction

$$P(\text{Sp}_N \rightarrow \text{Sp}_1) = \frac{\chi_{N-1} \cdot r_{(N-1)-} \cdot \Delta t}{[\text{Sp}_N]^t + r \cdot \Delta t/2} \frac{\chi_{N-2} \cdot r_{(N-2)-} \cdot \Delta t}{[\text{Sp}_{N-1}]} \cdots \frac{\chi_{i-1} \cdot r_{(i-1)-} \cdot \Delta t}{[\text{Sp}_i]} \cdots \frac{\chi_1 \cdot r_{1-} \cdot \Delta t}{[\text{Sp}_2]}$$

$$= \frac{\chi_{N-1} \cdot r_{(N-1)-} \cdot \Delta t}{[\text{Sp}_N]^t + r \cdot \Delta t/2} \prod_{i=N-1}^{2} \frac{\chi_{i-1} \cdot r_{(i-1)-} \cdot \Delta t}{[\text{Sp}_i]} \tag{A.12}$$

The ratio of $P(\text{Sp}_1 \rightarrow \text{Sp}_N)$ to $P(\text{Sp}_N \rightarrow \text{Sp}_1)$ is then

$$\frac{P(\text{Sp}_1 \rightarrow \text{Sp}_N)}{P(\text{Sp}_N \rightarrow \text{Sp}_1)} = \frac{\chi_1 \cdot r_{1+}}{[\text{Sp}_1]^t - r \cdot \Delta t/2} \frac{[\text{Sp}_N]^t + r \cdot \Delta t/2}{\chi_{N-1} \cdot r_{(N-1)-}} \frac{\prod_{i=2}^{N-1} \frac{\chi_i \cdot r_{i+}}{[\text{Sp}_i]}}{\prod_{i=N-1}^{2} \frac{\chi_{i-1} \cdot r_{(i-1)-}}{[\text{Sp}_i]}} \tag{A.13}$$

Rearranging,

$$\frac{P(\text{Sp}_1 \rightarrow \text{Sp}_N)}{P(\text{Sp}_N \rightarrow \text{Sp}_1)} = \frac{[\text{Sp}_N]^t + r \cdot \Delta t/2}{[\text{Sp}_1]^t - r \cdot \Delta t/2} \frac{\chi_1 \cdot \prod_{i=2}^{N-1} \chi_i \cdot r_{1+} \cdot \prod_{i=2}^{N-1} r_{i+} \prod_{i=N-1}^{2} [\text{Sp}_i]}{\chi_{N-1} \cdot \prod_{i=N-1}^{2} \chi_{i-1} \cdot r_{(N-1)-} \cdot \prod_{i=N-1}^{2} r_{(i-1)-} \prod_{i=2}^{N-1} [\text{Sp}_i]} \tag{A.14}$$

This equation can be further simplified to

$$\frac{P(\text{Sp}_1 \rightarrow \text{Sp}_N)}{P(\text{Sp}_N \rightarrow \text{Sp}_1)} = \frac{[\text{Sp}_N]^t + r \cdot \Delta t/2}{[\text{Sp}_1]^t - r \cdot \Delta t/2} \prod_{i=1}^{N-1} \frac{r_{i+}}{r_{i-}} \tag{A.15}$$

Comparing equations (A.5) and (A.15), we see that the ratio of the forward to reverse rates of the overall reaction can be expressed in terms of the ratios for the individual elementary steps as in equation (12).

Equation (12) was derived originally (Boudart, 1976) following a different and less concise procedure, from Temkin's identity equation (Temkin, 1971).

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