

The Role of Thermodynamics in Controlling Rumen Metabolism

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Abstract

Chemical reactions are controlled either thermodynamically or kinetically. Most mechanistic biological models assume kinetic control of metabolism pathways. The profile of products formed depends on substrate concentrations and enzyme activities that control the rates of synthesis for competing pathways. Thermodynamic control occurs when reactants are sufficiently limited relative to products that the reactions cannot proceed according to the Second Law of Thermodynamics. Under these circumstances, thermodynamics controls which pathway branches are available and the final concentration of products. This paper investigates the possibility of thermodynamic control of ruminal fermentation. Ruminal pH is buffered by bicarbonate in a system that is near equilibrium. This is a clear example of thermodynamic control. Rates of acetate, propionate and methane synthesis also appear to be controlled by thermodynamics, but these reactions do not approach equilibrium. These reactions proceed until the ΔG approaches a negative value representing the inherent inefficiency of the system. A dynamic model of glucose fermentation was developed that incorporated thermodynamic limits to VFA and gas production. Incorporation of thermodynamic limits was achieved by including fractional rate constants for reverse reactions. These reverse rate constants were determined from published thermodynamic data for the reactants and products. This model predicted realistic steady state concentrations of major VFA and gases. Incorporation of factors that decrease the efficiency of certain reactions (i.e. low pH or use of ionophores) would shift the fermentation in directions similar to those observed in the rumen.

Introduction

Ruminal fermentation initially results in the degradation of starch, fiber and protein to short-term intermediates such as sugars and amino acids. The kinetics of these initial degradation steps are frequently discussed, as they are considered rate-limited processes which are dependent on substrate level and enzyme specific activity, and are in turn affected by the microbial population and ruminal environment (Baldwin et al., 1987; Dijkstra et al., 1992). The products of this initial degradation are readily metabolized to microbial mass and carbon dioxide (CO₂), methane (CH₄), ammonia (NH₃) and volatile fatty acids (VFA): primarily acetate, propionate and butyrate and to a lesser degree, branched chain VFA and occasionally lactate (Prins, 1977). Baker and Dijkstra (1999) extensively reviewed what is known about the control of this secondary degradation but much remains to be discovered.

Murphy et al. (1982) developed a mathematical model based on the stoichiometry of major pathways in the rumen to predict relative proportions of VFA, CH₄ and CO₂ from digestible feed fractions: soluble carbohydrate, starch, hemicellulose, cellulose and protein. This model has since been used for many efforts of dynamic modeling (Baldwin et al., 1987; Dijkstra et al., 1992) with inconsistent levels of success (Neal et al., 1992; Kohn et al., 1995). The prediction is based on a principle component analysis of the data set, and not on a fundamental understanding of the factors that may alter VFA profiles. For this reason, although the model has been an essential element of whole animal modeling, it has not been flexible enough to predict VFA profiles of feeds that differ substantially from those in the data sets from which coefficients were derived. In addition, this approach provides little information as to how the ruminal fermentation may be manipulated by dietary factors other than those considered in the model's development, and provides little insight into other regulators of microbial growth such as ionophores.

A more mechanistic approach to predict profiles of VFA and gases from rumen fermentation requires an understanding of the control mechanisms of metabolism. Chemical reactions are controlled either thermodynamically or kinetically (Chang, 1981). If reactants are sufficiently limited relative to products then the reactions cannot proceed according to the Second Law of Thermodynamics. Under these circumstances, thermodynamics controls which pathway branches are available. On the other hand, if the rates of utilization of a substrate control the pathways for product formation where all of the reactions are thermodynamically favorable, the reactions are kinetically controlled. These rates may depend on substrate and/or enzyme concentrations.

Consider a system in which key intermediates are found in very low concentrations relative to products. For example, one would not expect to find appreciable concentrations of glucose in the rumen where fermentation occurs rapidly. This expectation results from our understanding that glucose is rapidly metabolized to other compounds. If the very moment a glucose molecule is released into solution it is a candidate for metabolism, whether that molecule becomes acetate, propionate, butyrate or lactate would depend on the speed of the respective reactions or at least on how quickly

the reactions can remove the molecule from solution. If the end products build up, however, the VFA may inhibit the reaction from occurring at all based on the Second Law of Thermodynamics. The Second Law of Thermodynamics requires that free energy (G) be released for a process to occur spontaneously. If the concentration of precursor becomes small enough relative to product the forward reaction will not proceed. In this case, the precursor may be used to produce a different product, and there may be a shift from one VFA or gas to another. Equilibrium thermodynamic principles were recently integrated into kinetic models explaining reaction shifts in anaerobic digesters (Hoh C., 1996).

The objective of the present paper is to demonstrate the potential for thermodynamic control of ruminal fermentation. This approach will allow determination of the possibilities for manipulating gas and VFA profiles and which pathway branches may control their production.

The Second Law of Thermodynamics

The First Law of Thermodynamics (that energy cannot be created nor destroyed but can be converted from one form to another) has been applied to animal biology since the 18th century. The First Law of Thermodynamics is applied when energy balance trials are conducted. The energy of fecal matter, urine, heat increment, maintenance, growth, and milk secretion is equal to the energy consumed in feed. The First Law of Thermodynamics is used to describe how much energy is in a feed and thus how much work can be done or how much milk and meat can be produced. The Second Law of Thermodynamics has been central to the fields of physics and chemistry, but for the most part, has not been incorporated into mathematical models of biological systems or explanations of occurrences in biology. The second law deals with how the energy will be used and whether or not the work will be done or whether the energy will be used for milk production, growth or heat.

The Second Law of Thermodynamics is: entropy of the universe increases in an irreversible process or remains unchanged in a reversible process (Chang, 1981). Entropy is defined as "randomness" and a reversible process is defined as an infinitely slow one with an infinite number of steps. In fact, most reactions are irreversible and so the entropy of the universe increases or heat is released from the system to the surroundings. Stated mathematically, the second law is as follows:

$$dS_u \geq 0 \quad \{1\}$$

where dS_u represents the change in entropy of the universe. This change in entropy can be divided between that of any given system (dS_{system}) and the surroundings of the system ($dS_{\text{surroundings}}$); therefore:

$$dS_u = dS_{\text{system}} + dS_{\text{surroundings}} \quad \{2\}$$

In a system that is imperfect, heat is generated while work is done. That heat is dissipated from the system to the surroundings. The system can be defined to include all changes in chemical composition of products and reactants. Thus only heat loss from the system needs to be considered as a loss to the surroundings. This heat loss ($-dH_{\text{system}}$) must equal the change in entropy of the system when corrected for temperature in degrees Kelvin (T).

$$dS_{\text{surroundings}} = -dH_{\text{system}} / T \quad \{3\}$$

By substituting the right hand side of equation 3 into equation 2, a new definition of the change in entropy of the universe is derived:

$$dS_u = dS_{\text{system}} - dH_{\text{system}} / T \quad \{4\}$$

Substituting the right hand side of equation 4 into equation 1 reminds us that the change in entropy of the universe must be greater or equal to 0.

$$0 \leq dS_{\text{system}} - dH_{\text{system}} / T \quad \{5\}$$

Multiplying both sides by (-T) provides the relation:

$$0 \geq dH_{\text{system}} - TdS_{\text{system}} \quad \{6\}$$

This negative value is the definition of the Change in Gibbs Free Energy (ΔG). Thus, ΔG of a system is negative when heat is lost from the system to the surroundings. The more inefficient the system is in using available energy, or the more heat lost, the more negative the ΔG must be. This relationship will be important to understanding shifts in ruminal metabolism associated with lower efficiency of fermentation and greater heat increment.

Integration of the Second Law of Thermodynamics with the Ideal Gas Law provides a mathematical relationship that is especially useful to chemistry and biology. Under constant pressure, the change in free energy of a reaction is a function of the activity of the products and reactants.

$$\Delta G = \Delta G^\circ + RT \ln\{[\text{Products}] / [\text{Reactants}]\} \quad (7)$$

where [x] represents activity of x (or pressure in atmospheres of x for ideal gases and molarity of x for ideal solutes), R is the gas constant and equals $8.314 \text{ J } ^\circ\text{K}^{-1} \text{ mol}^{-1}$, $^\circ\text{K}$ represents the temperature in degrees Kelvin, and ΔG° represents the change in free energy for the reaction under standard conditions. These conditions are $278.15 \text{ }^\circ\text{K}$, one unit of activity for all solutes and one atmosphere of pressure for all gases. If a reaction goes to equilibrium, the ΔG is equal to zero. Therefore, the ΔG° can be determined by measuring the concentration of products and reactants. Once this value is determined, it can be used with other similar values to determine the ΔG of other reactions contained

within these reactions. The amount of free energy of a material is intrinsic to that material. Furthermore, once the G is determined for potential reactants and products, the equilibrium constant for those reactions can be easily calculated. Setting ΔG to 0 in equation 7 represents equilibrium. Using book values for the ΔG° enables determination of the equilibrium constant (K_{eq}) as follows:

$$K_{eq} = e^{-\Delta G/RT} \quad \{8\}$$

Ruminal pH

One of the most obvious examples of thermodynamic control of ruminal metabolism is the control of pH by the bicarbonate ion (HCO_3^-). The bicarbonate system includes two major ionic forms: HCO_3^- and CO_3^{2-} . The HCO_3^- is of primary importance to buffering because it may be protonated to H_2CO_3 . The equilibrium constant for weak acid dissociation can be derived from setting the ΔG for the reaction to 0 and solving for the concentration of products over reactants. This value can be expressed as the negative log of the equilibrium constant, pK_a , to convert it to more convenient form. The pK_a of this acid is only 3.80 at 37°C and .15 M ionic strength (Segel, 1976). However, H_2CO_3 may establish an equilibrium with dissolved CO_2 and H_2O ,



The equilibrium constant (k_{eq}) for the two steps of the reaction combined is the product of the individual equilibrium constants.

$$K_{eq} = k_1 k_2 / k_{-1} k_{-2} = ([\text{HCO}_3^-] [\text{H}^+]) / ([\text{CO}_2] [\text{H}_2\text{O}]) \quad \{9\}$$

where k_1 and k_2 = fractional rate constants for each forward reaction, and k_{-1} and k_{-2} = fractional rate constants for each reverse reaction. Considering both steps in equilibrium, the effective pK_a for the system (pK_a') is 6.1 for solutions of .15 M ionic strength at 37°C (Segel, 1976). Ruminal gases and liquids are in close contact so that an equilibrium between soluble and evolved gas may be attained. The overall equilibrium constant for the ruminal system is therefore the product of three equilibrium constants for each of the reactions in which CO_2 gas is converted to HCO_3^- . The effective pK_a when determining $[\text{HCO}_3^-]$ for the rumen compared to the pressure of CO_2 in atmospheres (pCO_2) is 7.74. Thus the Henderson-Hasselbalch equation is as follows:

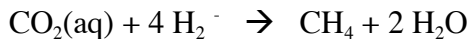
$$\text{pH}_{\text{rumen}} = 7.74 + \log ([\text{HCO}_3^-] / \text{pCO}_2) \quad \{10\}$$

The pH of ruminal fluid is clearly dependent on the concentration of HCO_3^- and the pressure of CO_2 . Some have argued that the rumen is saturated with CO_2 and so both of these variables are unchanging and uncontrollable. Kohn and Dunlap (1997) used equation 10 to demonstrate that changes in ruminal fluid pH result from one of three sources: 1) changes in strong ion (e.g. Na^+ , Cl^-) concentrations, changes in volatile fatty

acid (VFA) concentrations, and changes in pCO₂. Strong ion concentrations could be affected by diet, contribution from saliva, or even hot weather (ion excretion). VFA concentrations may result from different fermentation rates. Finally, the pressure of CO₂ in the rumen has been shown to vary substantially for fistulated cows (Barry et al., 1977). The rumen contains a large percentage of N₂ particularly after feeding which reduces the pCO₂ and increases pH. You could argue that the large percentage of nitrogen in rumen gases results from leakage around the fistula. Nonetheless, pH measurements do not usually account for the effect of pCO₂ on pH despite the large differences in pH these changing pressures have.

Methanogenesis and Reductive Acetogenesis

A second application of the Second Law of Thermodynamics is in the prediction of the feasibility of methane generation or synthesis of acetate from CO₂ and H₂. It is well understood that H₂ is used by methanogens to produce CH₄ which is thought to be a waste product of metabolism. If this H₂ could be used instead to generate acetate from CO₂, greater energy could be preserved for production. What is the feasibility of methanogenesis or reductive acetogenesis in the rumen under typical conditions? The balanced reactions of interest in this case are:



If the total gas pressure in the rumen approximates 1 atm, and CO₂ comprises 70% of that pressure the pCO₂ would be 0.7 atm. Multiplied by the solubility constant (Segel, 1976) for this ionic strength, temperature and pressure (.0229 mol / atm), this pCO₂ would provide 0.016 mol / L of dissolved (aq) CO₂ at equilibrium. The H₂ concentration can be calculated using the Nerst Equation from the reducing potential and pH (Segel, 1976).

$$\Delta E = \Delta E^\circ + R T / (n F) \text{ times } \ln ([\text{H}^+]^2 / [\text{H}_2]_g) \quad \{11\}$$

where ΔE is the reducing potential in volts measured using an appropriate electrode, ΔE° is the change in reducing potential for the reaction under standard conditions which is equal to zero for the H⁺ to H₂ half reaction, R is the gas constant (8.3145 J °K⁻¹ mol⁻¹), T is the temperature in °K, n is the number of moles reduced (2), and F is Faraday's constant (9.6487 x 10⁴ C / mol) which converts ΔE from ΔG. The solution for [H₂]_g in this case yields the concentrations under equilibrium conditions for the H₂ half reaction. Typical ruminal conditions are E = -0.315 V and pH = 6.5 (Barry et al., 1977). Thus, [H₂]_g would be 1.6 x 10⁻³ atm. For this example, let us assume that the partial pressure of CH₄ is 0.3 atm. Multiplied by its solubility constant (2 x 10⁻⁵; Fogg and Gerrard) yields a concentration of 6 x 10⁻⁶ mol / L. The molecular weight of water is 18 g/mol with 1000 g per L. Therefore, the molarity of pure water is 55.6 mol / L. Assuming 10% dry matter

of the ruminal solvent yields a molarity of approximately 50 mol / L for rumen liquid. Typical ruminal conditions allow for at least 0.05 mol / L of acetate. Thus the concentrations of all products and reactants for a particular set of ruminal conditions have been defined.

Table 1 shows key thermodynamic data under standard conditions for these reactants and products as well as some other important ruminal metabolites. These values represent the free energy of formation (ΔG_f°) and enthalpy of formation (ΔH_f°) of the metabolites from the elements (e.g. H_2 , O_2 , graphite). Free energy under standard conditions and concentrations, ΔG° can be determined from these tabular values for each reaction of interest (Chang, 1981):

$$\Delta G^\circ = \Delta G_f^\circ \text{ of products} - \Delta G_f^\circ \text{ reactants} \quad \{12\}$$

Adjustment to each ΔG_f° for temperature can be made using a transformation of the vant Hoff equation (Chang, 1981) and enthalpy of formation, ΔH_f° where T_1 and T_2 are the initial and final temperatures respectively, and $\Delta G_{T_1}^\circ$ and $\Delta G_{T_2}^\circ$ are the respective standard free energy values:

$$\Delta G_{T_2}^\circ = T_2 / T_1 [\Delta G_{T_1}^\circ - \Delta H^\circ (T_2 - T_1) / T_2] \quad \{13\}$$

Table 2 shows the resulting standard change in free energy (ΔG°) calculated for several reactions important to ruminal metabolism under standard conditions and adjusted for 311°K.

Now it is possible to use equation 7 to determine the ΔG of methanogenesis and acetogenesis under the ruminal conditions that were just described. For methanogenesis,

$$\Delta G = \Delta G^\circ + RT \ln \{ [CH_4]_{aq} [H_2O]^2 / ([CO_2]_{aq} [H_2]_g^4) \} \quad \{14\}$$

$$\Delta G = -134.9 + .008314 \times 311 \ln \{ (6 \times 10^{-6})(50^2) / [(0.016)(1.6 \times 10^{-3})^4] \} \quad \{15\}$$

$$\Delta G = -68.5 \text{ kJ / mol} \quad \{16\}$$

The ΔG for this reaction is negative, so the reaction is feasible. Now consider the ΔG for the use of H_2 for acetate production under the same ruminal conditions.

$$\Delta G = \Delta G^\circ + RT \ln \{ [C_3H_3O_2^-] [H^+] [H_2O]^2 / ([CO_2]_{aq}^2 [H_2]^4) \} \quad \{17\}$$

$$\Delta G = -72.2 + .008314 \times 311 \ln \{ 0.050 (1 \times 10^{-6.5}) 50^2 / [(0.016)^2 (1.6 \times 10^{-3})^4] \} \quad \{18\}$$

$$\Delta G = -10.4 \quad \{19\}$$

The ΔG is negative so the reaction is feasible under these conditions. However, the production of ATP was not considered for these reactions. In fact, including energy

for ATP production would have shifted the equilibrium so that more reducing conditions would be needed for acetogenesis.

Table 1. Standard free energy of formation and enthalpy of formation in kJ/mole of key rumen metabolites at 298.15°K and 1 atm.

Metabolite	ΔG°_f	ΔH°_f
□,□ D-Glucose (aq) (C ₆ H ₁₂ O ₆)	-916.97	-1263.78
Acetate (aq)	-376.89	-485.6
Propionate (aq)	-373.82	-511.70
Butyrate (aq)	-372.04	-535.55
Lactate (aq)	-516.72	-686.64
Methane (aq)	-50.79	-74.85
Carbon dioxide (aq)	-386.23	-412.92
Water (l)	-237.19	-285.84
Hydrogen (g)	0.0	0.0

Data are *not* adjusted to pH 7 and are from Chang, 1977 except for propionate (CRC, 1991). Standard conditions are 1 M concentration of each soluble reactant and product, 1 atm of all gases, and 298.15°K.

Thermodynamic Efficiency

For reactions that are very fast and inexpensive to catalyze (i.e. no complex enzyme systems are needed), the reactions can approach equilibrium. The pH buffers discussed earlier represent this type of reaction. However, complex biological pathways have built in inefficiencies so that it is not feasible for the reactions to proceed all the way to equilibrium. The efficiency of ATP synthesis can be determined from the concentrations of reactants and products. For example, the efficiencies of some key reactions in the rumen under typical conditions are shown in Table 3.

As expected, the efficiency for methanogenesis is higher than that for VFA production. Methane production is a simpler process than VFA formation so we would not be surprised to learn that less free energy is lost from carrying it out. Methanogenesis can occur at pH 7 and -0.33 V or more negative reducing potential (Wolfe and Higgins, 1979). An equivalent H₂ concentration would be observed at pH 6.5 and -0.30 V reducing potential. Thus, the conditions where methanogenesis can begin to occur are quite similar to the conditions in the rumen and methanogenesis appears to be thermodynamically limited.

The observed efficiencies for acetate and propionate production are probably near the maximal efficiencies that are feasible allowing for the necessary losses of heat from fermentation. Thus, the concentrations of acetate and propionate are probably limited by thermodynamics. On the other hand, maximal butyrate efficiency is probably higher than

Table 2. Key reactions in the rumen: standard change in enthalpy in kJ/mol (ΔH°), standard change in free energy at 298°K in kJ/mol (ΔG°_{298}), and standard change in free energy at 311°K in kJ/mol (ΔG°_{311}).

Reaction	Formula	ΔH°	ΔG°_{298}	ΔG°_{311}
Glucose to Acetate	$C_6H_{12}O_6 + 2H_2O \rightarrow 2C_2H_3O_2 + 2H^+ + 4H_2(g) + 2CO_2(aq)$	38.4	-134.9	-142.4
Glucose to Propionate	$C_6H_{12}O_6 + 2H_2 \rightarrow 2C_3H_5O_2 + 2H^+ + 2H_2O$	-331.3	-305.0	-303.9
Glucose to Butyrate	$C_6H_{12}O_6 \rightarrow C_4H_7O_2 + H^+ + 2H_2(g) + 2CO_2(aq)$	-97.6	-227.5	-233.1
Glucose to Lactate	$C_6H_{12}O_6 \rightarrow 2C_3H_5O_3 + 2H^+$	-109.5	-116.5	-116.8
Lactate to Propionate	$C_3O_3H_5 + H_2 \rightarrow C_3H_5O_2 + H_2O$	-110.9	-94.3	-93.6
Methanogenesis	$CO_2(aq) + 4H_2 \rightarrow CH_4(aq) + 2H_2O$	-233.6	-138.9	-134.9
Acetogenesis	$2CO_2 + 4H_2 \rightarrow C_2H_3O_2 + H^+ + 2H_2O$	-231.4	-78.8	-72.2
ATP generation	$ADP + Pi + H^+ \rightarrow ATP + H_2O$	24.3	-9.0	-10.4

Data are calculated from values in Table 1 except for ATP which is from Rekharsky (1986). Data are *not* adjusted to pH 7.

observed. Therefore, this pathway is probably not approaching the concentrations that would be infeasible and it is probably not dependent on product concentration. An efficiency greater than 1.0 is not possible even from the least complex of reactions, therefore reductive acetogenesis would not occur under these ruminal conditions.

The efficiency of lactate production is lower than that of acetate and propionate. Only two ATP are generated via lactate production and the lactate pathway to produce propionate is thought to produce less ATP than the direct pathway. These less efficient reactions are likely to proceed faster than the more efficient alternatives (Jou and Llebot, 1990). However, they would not be able to proceed at all unless the concentration of reactants relative to products was high. Thus, it is likely that lactate cannot be produced even at the low efficiencies shown here and would not exist under these conditions. If the glucose and VFA concentrations increased (due to a momentary increase in fermentable energy or inhibition of pathways by low pH), lactate synthesis would play a role in rapidly using the available nutrients. Once the microbial population adapted, more propionate producers could compete and reduce glucose levels to again limit lactate production.

Use of Thermodynamics in Dynamic Models

Judging from the previous analysis of the efficiencies of VFA production, it seems appropriate to incorporate thermodynamics into kinetic and dynamic models of fermentation. Acetate, propionate, methane and lactate all seem to be limited by

Table 3. Free energy of key reactions in the rumen in kJ / mol under typical ruminal conditions.

Reaction	No. ATP	ΔG_{Rxn}	ΔG_{ATP}	ΔG_{Total}	$-\Delta G_{ATP} / \Delta G_{Rxn}$
Glucose to Acetate	4	-317.7	177.5	-140.2	0.559
Glucose to Propionate	4	-321.8	177.5	-144.3	0.552
Glucose to Butyrate	3	-312.0	133.1	-178.9	0.427
Glucose to Lactate	2	-206.1	88.8	-117.3	0.431
Methanogenesis	1	-67.9	44.4	-23.5	0.653
Acetogenesis	0.2	-8.7	8.9	0.1	1.015
ADP to ATP	1	44.4	---	---	---

Data are calculated from values in Table 3 and assuming the following ruminal conditions: [glucose] = 0.1 mmol / L, [acetate] = 80 mmol / L, [propionate] = 30 mmol / L, [butyrate] = 30 mmol / L, [lactate] = 1 mmol / L, [CH₄] = 0.3 atm, [CO₂] = 0.7 atm, [H₂O] = 50 mol / L, [H₂] = .00152 atm, [ATP] = 1 mmol / L, [ADP][Pi] = .002 mmol / L, and pH = 6.5.

thermodynamics at some time. Dynamic models need to account for these limits so that infeasible concentrations are not predicted. Including thermodynamics will make the models more stable.

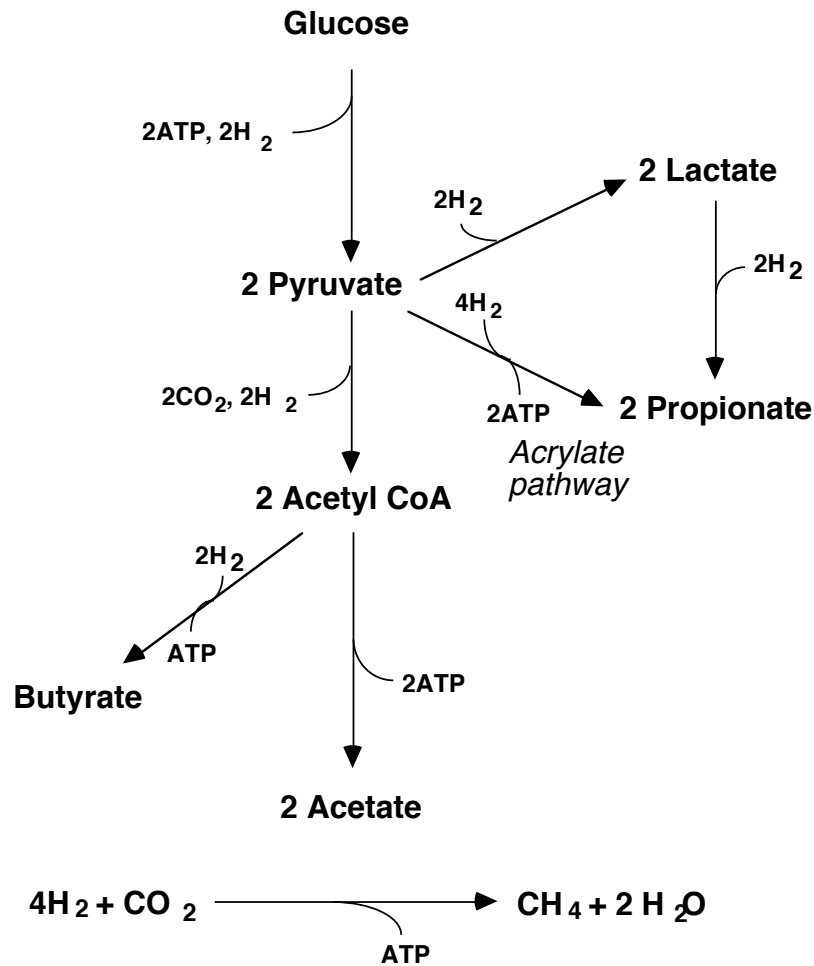
Previously, we calculated the efficiencies of free energy utilization for specific metabolite concentrations. Development of dynamic models using thermodynamics requires estimation of the maximum potential efficiencies. The actual efficiency may be lower unless the reactions approach thermodynamic limits. The ΔG at the maximum efficiency is called the threshold free energy (ΔG_T). It is the ΔG of a reaction when that reaction is as close as it can ever get to zero but for reactions with built in inefficiency it is always negative. The ΔG_T is equivalent to the unavoidable losses in free energy (i.e. heat loss from a system) and it can be calculated from a modification of equation 7:

$$\Delta G_T = \Delta G^\circ + RT \ln\{[\text{Products}]_T / [\text{Reactants}]_T\} \quad \{20\}$$

Where $[X]_T$ represents the concentrations of reactant or product X when the system approaches its threshold. When a reaction approaches the Threshold ΔG , the forward reaction rate equals the reverse reaction:

$$k_{rxn} [\text{Reactants}] = k_{-rxn} [\text{Products}] \quad \{21\}$$

Figure 1. Major fermentation pathways in the rumen (adapted from Prins, 1977).



where K_{rxn} and K_{-rxn} represent the fractional rate constants. Combining equations 20 and 21 yields a ratio that is analogous to an equilibrium constant for reactions that never obtain equilibrium due to the inherent inefficiencies and complexities of the system:

$$k_{rxn} / k_{-rxn} = e^{(\Delta G^\circ - \Delta G^\circ T) / RT} \quad \{22\}$$

A dynamic model of glucose fermentation was developed assuming the stoichiometry shown in Figure 1 and assuming 56% efficiency of glucose fermentation to acetate, propionate or butyrate and 70% efficiency of methane production. These efficiencies were used to represent the highest efficiencies typically observed for the rumen. The ΔG_T was determined from these assumed efficiencies, and from these values the k_{rxn} / k_{-rxn} was determined for each reaction. Initial concentrations of each metabolite were set to approximate values that were similar to those observed in the rumen. Forward rate constants were set to values such that when multiplied by each of the reactant concentrations, the resulting fluxes were realistic. The reverse rate constants were calculated as the K_{rxn} (as set previously) divided by the K_{rxn} / K_{-rxn} . Glucose was infused continuously into this system at a rate of 25 mmol h⁻¹ L⁻¹ and VFA were removed at a fractional rate of 0.40 / h. As gas pressures exceeded 1 atm, CO₂ and CH₄ were allowed to escape in the proportions that they were predicted to represent in the gas phase.

The results of the simulation to steady state are shown in Table 4. Wolin (1960) showed using fermentation balances that the stoichiometry of the rumen is balanced but this solution did not explain the unique combination of metabolites in the rumen. For example, a fermentation of only butyrate and methane can be balanced stoichiometrically but such a fermentation does not exist. The inclusion of thermodynamics explains the proportions of each VFA and gas that are observed.

The dynamic model can be used to show how ionophores affect ruminal fermentation patterns. Ionophores result in reduced methane, increased propionate and higher pH. They permit ions to penetrate gram negative bacteria such as the acetate producers (Russell and Strobel, 1989). This effect should increase the cost of acetate production by causing the gram negative organisms to expend ATP to repair internal ion concentrations. This cost would directly decrease the threshold ΔG (make more negative) for acetate and would shift the equilibrium against acetate production. The glucose spared from this shift would further increase propionate production, which would decrease H₂ available for methane.

As the energy density of the diet increases, acetate to propionate ratio and methane production decline while H₂ concentration increases. Since methane does not increase in response to the increased H₂, the calculated efficiency for methanogenesis would decrease. This change suggests that the ruminal conditions of high energy diets make it more energetically expensive to produce methane causing the efficiency to decline. This change would favor propionate production over methane production. Therefore, the thermodynamics suggests that methanogenesis is inhibited on high energy diets thus causing the shift to propionate (Kohn and Boston, 1995). Recent research supports this hypothesis by suggesting that methanogenesis appears to be inhibited by low pH (Russell, 1998) which could result from rapid fermentation. However, another mechanism could also explain the shift in equilibrium toward propionate. The efficiency of acetate production could also decrease along with the efficiency of methanogenesis, which would also shift the fermentation toward propionate.

Up until now, microbiologists have preferred to use anthropomorphic explanations to explain fermentation shifts. For example, certain organisms are thought

to prefer certain substrates and make certain products. But these explanations do not explain why certain organisms would shift their fermentation or thrive in certain environments. The use of the Second Law of Thermodynamics explains these shifts more systematically as being caused by changes in the ruminal environment.

Table 4. Predicted steady state concentrations and fluxes for a mathematical model incorporating thermodynamic and kinetic elements.

Metabolite	Concentration		
Glucose, mmol / L	0.15		
Acetate, mmol / L	59.1		
Propionate, mmol / L	38.6		
Butyrate, mmol / L	13.8		
CH ₄ , atm	0.31		
CO ₂ , atm	0.69		
H ₂ , atm	1.3 x 10 ⁻³		
Acetate / Propionate	1.5		

Reaction	Flux (mmol substrate used per hour)		
	<u>Forward</u>	<u>Reverse</u>	<u>Net</u>
Glucose → 2 Acetate	15.2	3.6	11.6
Glucose → 2 Propionate	11.9	4.2	7.7
Glucose → Butyrate	5.4	10 ⁻¹⁶	5.4
CO ₂ → CH ₄	14.4	3.6	10.8

Modle assumed 56% maximal free energy efficiency for acetate, propionate and butyrate formation, and 70% maximal efficiency for methane formation, pH 6.5, glucose infusion at 25 mmol L⁻¹ h⁻¹, fatty acid removal at 0.40 / h and gas removal as accumulated in proportion to partial pressures.

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