

The thermodynamics of growth of *Escherichia coli* K-12 on succinic acid

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Abstract

Postulates are presented which may help to standardize the construction of equations representing heterotrophic catabolism and anabolism, and the subsequent use of these to calculate the thermodynamic changes accompanying the growth processes they represent. These postulates are applied to the growth of *E. coli* K-12 on one mol of succinic acid. The $\Delta_r G'$, $\Delta_r H'$, and $\Delta_r S'$ accompanying anabolism are calculated to be -4.16 kJ, 5.05 kJ, and 30.89 J/deg, respectively, indicating that these energy changes are quite small, and that those accompanying the growth process are due almost entirely to catabolism.

The assumptions made and the methods used to construct equations for heterotrophic growth processes and to calculate the thermodynamic changes accompanying the growth processes they represent are not standardized in the literature at the present time. The following postulates may be useful in this respect. Many of these are self evident; others come from observations or suggestions in the literature. The term "growth process" adopted by Battley (ref.1) is used to acknowledge the fact that growth is not a single reaction, but an extremely complex set of reactions that would better be considered a "process." The word "process" applies equally to anabolism, catabolism, or metabolism. The term "fabric" of a cell was adopted by Battley (ref.2, p.1930) by analogy with the architectural term denoting the structural part of a building. It is used here to represent the permanent structure of the cell including enzymes in solution within the living cell. The terms "unit carbon formula" and "unit carbon formula weight" are abbreviated UCF and UCFW, respectively. The term "unit carbon formula weight equivalent" is abbreviated UCFW eq. and represents the UCFW plus the weight of ions found in the cells equivalent to that contained in one UCFW of living cells. The postulates apply to microbial batch culture systems in which defined media are used, and are as follows:

1. It is possible intellectually to separate a growth process into anabolism and catabolism. This postulate has not been challenged seriously since 1886, when Gaskell (ref.3, p.46) first used these words to represent the concepts (see also ref.1). Heijnen and van Dijken (ref.4) have pointed out that the method of splitting a growth process into anabolism and catabolism is always arbitrary. Any arbitrary split is permitted by Hess's Law provided that the equations representing anabolism and catabolism sum up to the same equation representing the given growth process. However, the most correct split is likely to be the one that most accurately describes the overall biochemistry of what is happening during anabolism and catabolism.

2. The thermodynamic systems initially conceived should be as simple as possible. This postulate is based on the idea ascribed to William of Ockham (ref.5) that "Essentia non sunt multiplicanda praeter necessitatem." ("Essentials ought not to be multiplied except of necessity.").

3. The simplest system to study involving microbial growth is that in which the initial state comprises a culture flask containing a defined medium containing appropriate minerals, trace elements, and vitamins; in which the nitrogen source is an ammonium salt; and in which there is a small quantity of a single organic source of carbon and energy. The mass of the inoculum can be considered negligible. The final state comprises the culture flask and its contents after growth has ceased due to the exhaustion of the substrate, which is the only limiting factor. Ideally, the quantity of substrate is no more than that which can be oxidized by the dissolved oxygen present in the medium, and from which any carbon dioxide produced will remain in solution. The pressure of the system then remains constant. The temperature should be that at which the microorganisms grow at μ_{\max} . Battley (ref.6, p.335) pointed out that under these conditions of batch culture no storage materials are formed within the cells. This is indicated by the calorimetric work of Dermoun and Belaich (ref.7, p.378) with *E.coli* K-12 grown on succinic acid, showing that the production of heat stops immediately and completely when the substrate has disappeared.

4. Storage substances are not a part of the fabric of a cell. This was first emphasized in 1900 by Duclaux (ref.8, p.328), who pointed out that in yeast "...le glycogen, aliment interieur, remplace pendant quelques temps le sucre, aliment exterieur, lorsque celui-ci a disparu." ("... glycogen, an interior food substance, replaces sugar, an exterior food substance, when this [latter] has disappeared."). Any microbial storage product can be used as a source of carbon and energy, and since it can disappear from the cell, it does not constitute a part of the permanent cellular structure, or fabric. Cells not containing storage substances result from being grown exponentially at μ_{\max} . Suspensions of cells placed in a medium containing a carbon and energy source but no nitrogen source will assimilate the substrate and form storage substances, but cannot grow (ref.1, Chapter 5). Under these circumstances, when μ is zero, the cells form a maximum quantity of storage products and exhibit a substantial increase in dry weight. Intermediate conditions apply during growth in continuous culture, when μ can be regulated to be anything between zero and μ_{\max} . Under these conditions the cells will form more or less storage product as a function of the difference between the rate at which the cells can assimilate the substrate and the rate at which growth actually occurs. The true extent of growth can then be determined only by subtracting the mass of the storage material from the biomass of the cells as a whole. This is seldom done, but should be considered seriously. For example, Battley (ref.1, p.393) pointed out with respect to the growth of *Saccharomyces cerevisiae* on glucose, that the oxidative assimilation of only 20% of the glucose, resulting in the synthesis and storage of glycogen, would result in an increase in dry weight of 52.1% over what would occur if growth were the only process taking place. This gives an erroneous measurement of the apparent increase in dry weight resulting from growth.

5. Cells are precipitates, and the fabric of the cell should be treated thermodynamically as if it were a solid (Battley, ref.9, p.1934). This includes the assumption that the energy involved in the hydration of the cellular fabric is negligible, which has yet to be tested.

6. The water matrix within the cells is considered to be inert thermodynamically. This idea was first implicated in 1955 by Morowitz (ref.10, p.82), who considered "...the nonaqueous portion of the cell...as constituting the basic information unit necessary to specify a living system."

7. The substances participating in anabolism and catabolism, except for the cells, should be treated thermodynamically as if they are in the aqueous state at a real concentration of 0.001 *m* and unit activity. This originates from the work in 1928 of Fulmer and Liefson (ref.11). The standard method of calculating the free-energy changes accompanying irreversible biochemical reactions is to determine the difference between the sums of the free energies of formation of the reactants and the products, all in the aqueous standard state, with the provision that the concentra-

tion of each reactant and product remains constant at 1 *m*. In batch cultures of microorganisms this does not occur. Fulmer and Liefson pointed out that if a microbial culture is allowed to proceed until the substrate becomes exhausted, the substrate concentration does not remain at the initial concentration, but will become diluted as it is consumed, whereupon its free energy of formation per unit mass will become more negative. The reverse applies to the products of microbial growth that are in solution. The free energy of formation of a solute under these conditions is then;

$$\Delta_f G' = \Delta_f G^\circ(\text{aq}) + [(-RT/1000) \ln \frac{1}{a_c}]; \text{ (see Battley, ref.1, p.374)}$$

where the values for $\Delta_f G'$ and $\Delta_f G^\circ(\text{aq})$ are in kJ/mol, and a_c represents the activity of a solute at a concentration, *c*, which is less than 1 *m*. As soluble substrate becomes consumed and a_c becomes sufficiently small the value of its free energy of formation approaches minus infinity. This also applies to the products of a growth process in the initial state, when they have a concentration of zero. Fulmer and Liefson attempted to solve this problem with respect to their calculations of the free energy change accompanying the alcoholic fermentation by yeast of sucrose by assuming small, finite values for the concentrations of reactants and products. For example, the atmospheric concentration of carbon dioxide was taken as that in equilibrium with the aqueous medium. They also accepted an average $\Delta_f G'$ with respect to the highest and lowest concentrations of any given solute, thus effectively integrating the $\Delta_f G'$ values. Another solution to this problem was provided by Battley (ref.1, p.374), who suggested that a new "practical" standard state be adopted. He pointed out that at concentrations of 0.001 *m*, activities and concentrations are for most purposes equal. Under these conditions the "biological" standard state becomes a real 0.001 *m* concentration having the same activity, and the $\Delta_f G'$ values for solutes become more negative by 17.11 kJ/mol.

8. Ions within the cells should be treated thermodynamically as if they are present in the aqueous state at a real concentration of 0.001 *m* and the same activity. The reasons for this are partly those covered in postulate 7, above, and partly because ions are required in much lower quantities than the substrate. The thermodynamic properties of ions can be regarded as being the same both outside and inside of the cells, since in the latter they are dissolved in the aqueous matrix that supports the fabric of the cells.

9. The construction of an equation representing anabolism should be made using electron equivalences, not carbon mol equivalences. In this manner differences between the state of oxidation of the carbon and energy substrate and that of the cellular substance are accounted for in the anabolic reaction by an exchange of CO₂ rather than O₂. Thus, dioxygen does not participate in anabolism, and should not be present in an equation representing anabolism (Battley, ref. 1, p.398). Oxygen serves primarily as a final electron acceptor. Its principal function in anabolism was established by Maguigan and Walker (ref.12), who showed that it is required for steroid synthesis. On the other hand, the requirement for oxygen in steroid biosynthesis as it is presently understood for eukaryotes is sufficiently small that its mass can be ignored with respect to anabolism. Prokaryotes do not possess steroids and do not require oxygen for this purpose. Also, cellular growth processes presumably evolved before oxygen was present on the surface of the planet and therefore must have been anaerobic. Dioxygen would not have been available for anabolism during this period.

10. The representation of anabolism with respect to exponentially grown microorganisms is the same whether the conditions of growth at u_{max} are aerobic or anaerobic. This follows when electron equivalences are used in the construction of the anabolic equation.

11. The process of growth is irreversible.

12. The thermodynamic input into a growth process system is the non-thermal energy present in the substrate in the initial state. The output is the thermal energy released in the form of enthalpy or exchanged as entropy.

13. No maintenance energy is required during the growth process. This is discussed in detail by Battley (ref.1, p.410).

14. The per cent efficiency of free energy conservation is equal to:

$$\frac{\Delta_r G'_i - \Delta_r G'_{\text{met}}}{\Delta_r G'_i} \times 100$$

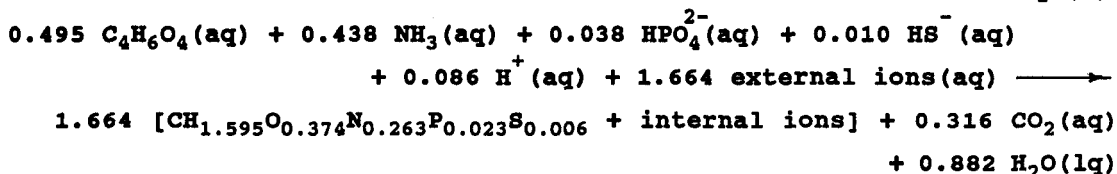
where $\Delta_r G'_i$ represents the free energy that is available in the initial state for the complete fermentation or oxidation of the substrate, and $\Delta_r G'_{\text{met}}$ represents the free energy change accompanying metabolism.

15. There is no net work done during the process of microbial growth, other than the very small amount of PV work that may be done during the expansion of metabolic gases against the atmosphere, or mechanical work exerted during microbial movement.

Using some of the above postulates, Battley (ref.6) adopted the following set of equations representing the anabolism, catabolism, and growth process of *E.coli* K-12 on succinic acid.

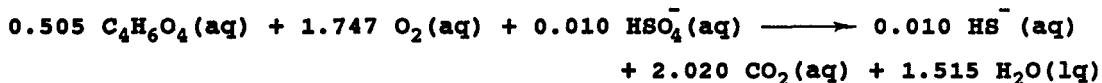
ANABOLISM:

Eq. (1)



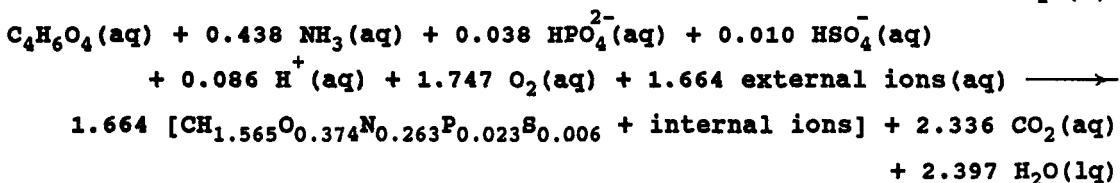
CATABOLISM:

Eq. (2)



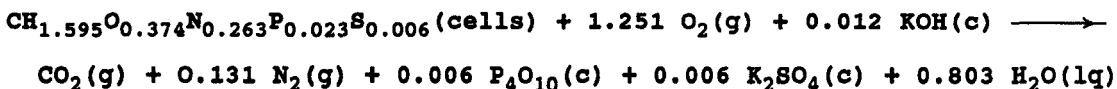
GROWTH PROCESS = METABOLISM:

Eq. (3)



Battley (ref.13) constructed the following equation representing the combustion of the cells, in which the K comes from the complement of ions within the cells. It is assumed that the thermodynamic state of each ion is the same both before and after combustion. These ions do not therefore contribute to any changes in the free energy, enthalpy, and entropy of the system during the combustion.

Eq. (4)



Patel and Erickson (ref.14) used two methods of determining the heat of combustion of organic substances per electron equivalent transferred to oxygen, $\Delta H_c/\text{eq.}$, one being a linear regression analysis with the line passing through the origin, and the other being a statistical analysis

resulting in an average value. According to Erickson (15), there is no reason to accept one method as being "better" than another. Values obtained by the two methods are therefore averaged, giving -110.88 kJ/eq. (regression analysis) + $(-111.40 \text{ kJ/eq. (average)})/2 = -111.14 \text{ kJ/eq.}$, this value being within one per cent of whatever the "best" value happens to be. Battley (ref.13) calculated that one UCFW of *E. coli* K-12 cells contains 4.998 electrons relative to bomb calorimetric combustion, making the heat of combustion of this quantity of cellular material $4.998 \text{ eq./UCFW} \times -111.14 \text{ kJ/eq.} = -555.47 \text{ kJ/UCFW}$. Patel and Erickson used only organic substances containing C, H, O, and N in their determination, whereas dried cells contain in addition P and S. The P in cells is already in its highest oxidation state. Cellular protein sulfur is present as the sulfide, for which the heat of combustion per equivalent is -99.17 kJ , rather than -111.14 kJ . However, this is not a large difference and because the quantity of sulfur in cells is so small, this difference can be neglected. Using Eq.(4) and the above heat of combustion together with $\Delta_f H^\circ$ values for the reactants and products from Weast (ref.16, p.D52-D93), the $\Delta_f H/\text{UCFW}$ of cells is calculated to be -88.99 kJ . Adding the $\Delta_f H^\circ$ value of -6.69 kJ for the ions (Battley, ref.6) gives $-95.68 \text{ kJ/UCFW eq.}$ Using this in Eq.(1) representing anabolism together with the appropriate $\Delta_f H^\circ$ values for the reactants and products from Battley (ref.13) and Wilhoit (ref.17) the enthalpy change accompanying anabolism is calculated to be 5.05 kJ , a value which is close to zero. This result is in agreement with the observation in 1922 by Molliard (ref.18) that the heat of growth of *Sterigmatocystis nigra* was due entirely to catabolism. The heat of combustion value of $-555.47 \text{ kJ/UCFW eq.}$ is very close to that of -548.55 ± 27.77 obtained by averaging ten combustion values for *E.coli* obtained from the literature, as shown in Table III of ref.13. This indicates that the value of -111.14 kJ/eq. can be used with considerable confidence in calculating heats of combustion of dried cells.

The values for the free energy change accompanying the bomb calorimetric oxidation of organic material were determined by Erickson and Patel (ref.19) to be -107.06 kJ/eq. (regression analysis) and -108.74 kJ/eq. (statistical analysis), an average of which is -107.90 kJ/eq. This value multiplied by 4.998 eq./UCFW gives -539.28 kJ/UCFW as the free energy change, ΔG_c , accompanying bomb calorimetric combustion. Using this value in Eq.(4) together with $\Delta_f G^\circ$ values for the reactants and products from Weast (ref.16, p.D52-D93), the $\Delta_f G/\text{UCFW}$ of cells is found to be -65.10 kJ . Adding to this the $\Delta_f G^\circ$ value of -6.92 kJ for the ions (Battley, ref.19) gives $-72.02 \text{ kJ/UCFW eq.}$ Using this in Eq.(1) representing anabolism together with the appropriate $\Delta_f G'$ values at an activity of 0.001 for the reactants and products from Battley (ref.20), the $\Delta_f G'$ accompanying anabolism is calculated to be -4.16 kJ . This is close to zero and is in agreement with the hypothesis of Battley (refs.1 and 21) based on completely different data, that the free energy change accompanying the growth of cells is due almost entirely to catabolism. Using the Gibbs free energy equation and the above values for $\Delta_f H'$ and $\Delta_f G'$, the $\Delta_f S'$ accompanying anabolism per mol of succinic acid utilized is calculated to be $\{[5.05 \text{ kJ} - (-4.16 \text{ kJ})] \times 1000\}/298.15 \text{ K} = 30.89 \text{ J/deg}$, which is also close to zero. Again using the Gibbs free energy equation, the value for $\Delta_f S'$ is calculated to be $\{[(-95.68 \text{ kJ/UCFW eq.}) - (-72.02 \text{ kJ/UCFW eq.})] \times 1000\}/298.15 \text{ K} = -79.36 \text{ J/UCFW eq. deg}$. The entropy of these cells can be calculated as follows:

$$\text{Eq. (5)}$$

$$S' = \Delta_f S' + 5.740nC + 65.287nH + 102.514nO + 95.751nN + 41.087nP + 31.798nS$$

In Eq.(5) S' represents the entropy of a given mass of an organic substance, here, one UCFW eq. of *E.coli* K-12 structural fabric. Superscript $^\circ$ notations for S' or $\Delta_f S'$ are not used because dried cells are not pure substances, otherwise the conditions are standard. The constants 5.740, 41.087, and 31.798 are the entropies of solid graphite, white phosphorous, and rhombic sulfur, respectively. The constants 65.287, 102.514, and 95.751 are one-half the entropies of $\text{H}_2(\text{g})$, $\text{O}_2(\text{g})$, and $\text{N}_2(\text{g})$, respectively, all at 298.15 K and one atm and all having the dimensions of J/g

atom deg. Using Eq.(5), the above value for $\Delta_f S'$, and the UCF from Eq. (1), the entropy of one UCFW of dried *E.coli* cells is calculated to be 95.17 J/UCFW deg. Using the appropriate data from refs. 16, p.D52-D93, and 20, the $\Delta_r G_i'$ accompanying the complete oxidation of the substrate to $\text{CO}_2(\text{aq})$ and $\text{H}_2\text{O}(\text{lq})$ is calculated to be -1557.56 kJ/mol. Similarly, using Eq.(3) $\Delta_r G_{\text{met}} = -783.07$ kJ/mol. From postulate 14 the free energy conserved is then $[-1557.56 - (-783.07)] \times 100 / -1557.56 = 49.72\%$.

LIST OF NON-STANDARD SYMBOLS NOT OTHERWISE DEFINED

- $\Delta_f G^{\circ'}$, $\Delta_f H^{\circ'}$, $\Delta_f S^{\circ'}$, free energy, enthalpy, or entropy of formation, respectively, of a specified quantity of a pure substance in the "biological" standard state in aqueous solution at an activity of 0.001 and 298.15 K and 1 atm.
- $\Delta_f G$, $\Delta_f H$, $\Delta_f S$ free energy, enthalpy, or entropy of formation, respectively, of a specified quantity of an impure, condensed substance not having a standard state at 298.15 K and 1 atm. An example of this would be dried biomass.
- $\Delta_f G'$, $\Delta_f H'$, $\Delta_f S'$ free energy, enthalpy, or entropy of formation, respectively, of a specified quantity of an impure, condensed substance not having a standard state at 298.15 K and 1 atm but suspended in the aqueous phase.
- $\Delta_r G$, $\Delta_r H$, $\Delta_r S$ free energy, enthalpy, or entropy change, respectively, accompanying a reaction of substances at least one of which does not have a standard state at 298.15 K and 1 atm.
- $\Delta_r G'$, $\Delta_r H'$, $\Delta_r S'$ free energy, enthalpy, or entropy change, respectively, accompanying the reaction of substances at least one of which does not have a standard state at 298.15 K and 1 atm, but is suspended or dissolved in an aqueous environment.

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